INVESTIGATION OF METHODS FOR STERILIZATION OF POTTING COMPOUNDS AND MATED SURFACES

George C. Marshall Space Flight Center
National Aeronautics and Space Administration
Marshall Space Flight Center, Alabama 35812

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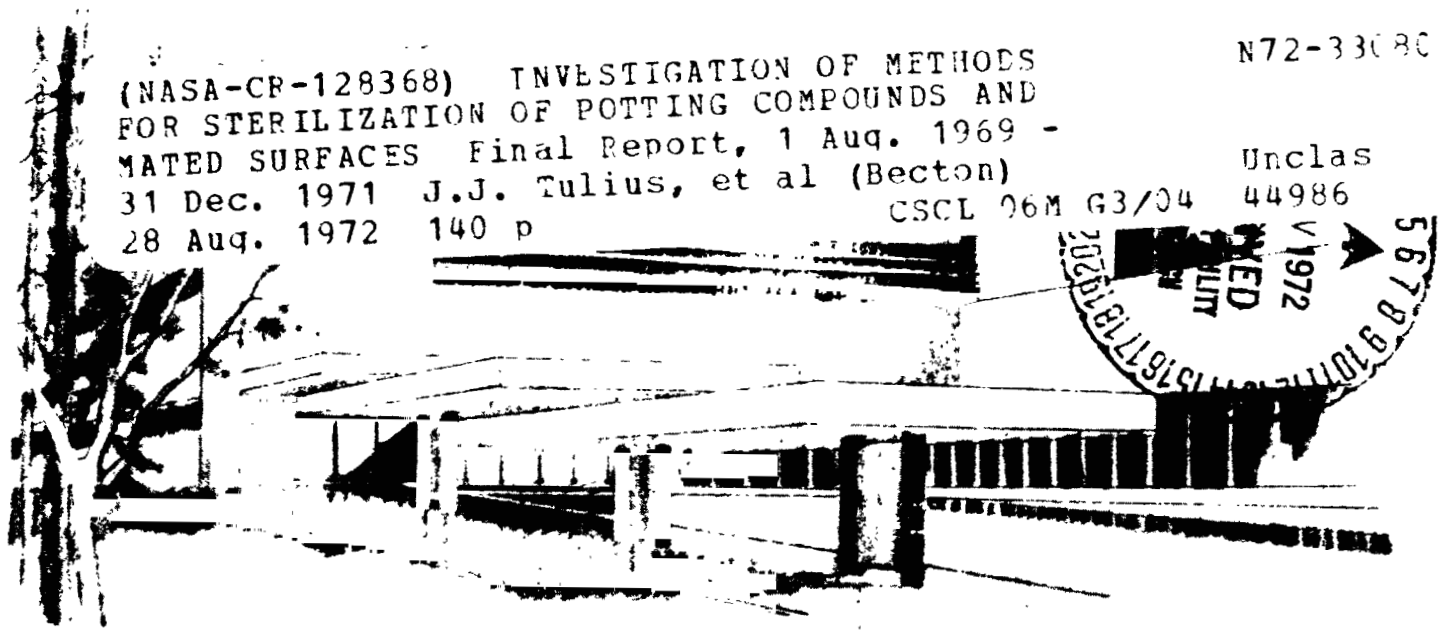

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FINAL REPORT

Contract # NAS8-24513

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(For Period of 8/1/69 through 12/31/71)

Prepared For

George C. Marshall Space Flight Center
National Aeronautics & Space Administration
Marshall Space Flight Center, Alabama 35812

August 28, 1972

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INTRODUCTION

This report presents the research conducted by Becton, Dickinson and Company during the period of August 1, 1969, through December 31, 1971, in support of the planetary quarantine requirements of the National Aeronautics and Space Administration. Research management was initially provided under Contract NAS8-24513 by Dr. G. Briggs Phillips, Director, Becton, Dickinson Research Center (BDRC), and Dr. J. J. Tulis, Director, Microbiological Sciences Department, BDRC, acting as Project Officer and Assistant Project Officer, respectively. During the extension of the contract work, research management was provided by Dr. J. J. Tulis and Dr. D. J. Daley, Research Associate, Microbiological Sciences Department, BDRC, acting as Project Officer and Assistant Project Officer, respectively. The subject research program was conducted as a continuation of the previous one-year contract with NASA Headquarters (NASw-1764) and was funded by Marshall Space Flight Center (MSFC), Huntsville, Alabama.

Technical briefings on the status of the contract research were presented at the NASA Spacecraft Sterilization Technology Seminars held at Las Vegas, Nevada, in September, 1969; Atlanta, Georgia, in May, 1970; Williamsburg, Virginia, in December, 1970; and Seattle, Washington, June, 1971.

Previous studies conducted under Contract NASw-1764 demonstrated the feasibility of internal sterilization of electronic potting compounds using formaldehyde-liberating synthetic resins and polymers. Research carried out from ambient to 125C indicated that the polymer, paraformaldehyde, was considerably more effective as a sterilizing agent than were the formaldehyde resins urea formaldehyde, melamine formaldehyde, or phenol formaldehyde.

Experimental verification for internal sterilization of potting compound was obtained using a spore-embedding technique developed for the purpose. The potting compound RTV-3140 (Dow Corning) was used as a representative electronic material for these studies since it possessed a number of characteristics which made it very convenient for embedding and recovering test organisms. Preliminary studies were also conducted using dimethyl sulfoxide (DMSO) in conjunction with monomeric formaldehyde and results indicated that sporicidal activity was enhanced using this combination.

The successful completion of Contract NASw-1764 led systematically to the program reported herein. These data are concerned with the results of a screening program conducted to select candidate carrier materials from various flight approved compounds, sterilant additives from various formaldehyde bleaching resins and polymers, and candidate test systems for verification of internal sterility of materials and surface sterility of mated components. In addition, studies included an evaluative program on the sterilizing efficiency of model sterilant-carrier systems as a function of time and temperature, additive concentration, moisture and penetrability (i.e., of mated and occluded surfaces). During the past year's extension, studies were conducted on the sterilization of bacterial spores on exposed and barrier-enclosed surfaces as a function of preconditioning and exposure levels of humidity and temperature at low concentrations of formaldehyde. As a part of this study a number of trials were conducted on the cold sterilization of exposed surfaces of a spacecraft mock-up housed within a 24 foot diameter chamber at MSFC.

This report documents all of the in-house and field trials conducted during the course of the original contract (NAS8-24513) and its extension and is submitted as a final report on the completed contract work.

SUMMARY

The experimental studies which have been completed during the period of this contract effort have culminated in the following pertinent findings:

- feasibility was established for addition of formaldehyde-liberating chemical sterilants to selected potting compounds, sealants, and adhesives.

- further evidence was obtained which confirmed that the polymeric form of monomeric formaldehyde, i.e., paraformaldehyde, was considerably more effective as a sterilizing agent than were the synthetic resins.

- experimental verification for internal sterilization was obtained by employing a spore-embedding technique developed for this purpose.

- results indicated that although the various sterilant additives tested (i.e., paraformaldehyde, melamine formaldehyde, and urea formaldehyde) contained the same amounts of available formaldehyde residue, significant differences in the thermal rate of release provided grossly different sterilant systems.

- the feasibility of sterilizing occluded areas of spacecraft components has been demonstrated with the use of a paraformaldehyde-trichloroethylene spray.

- potting compound-sterilant and lucite-sterilant mixtures proved inhibitory against both Gram positive and negative microorganisms.

- indirect evidence was obtained from studies on the loss of formaldehyde from potting compound-sterilant and lucite-sterilant

mixtures which suggested that internal sublimation of sterilants with outward diffusion of vaporized formaldehyde transpired.

- the screening program for selection of candidate carrier materials from various flight approved potting compounds, sealants, and adhesives provided by MSFC was completed.

- quantitative studies on the inactivation of bacterial spores embedded in RTV-3140 to which 1% paraformaldehyde had been added indicated that curing for 10 to 12 days at ambient temperature was sufficient to internally sterilize the potting compound (i.e., kill 10^5 spores).

- quantitative inactivation studies conducted with spores embedded in RTV-3140 and Chem Seal 3547 confirmed that paraformaldehyde was the most effective source of monomeric formaldehyde followed by urea formaldehyde and melamine formaldehyde, respectively.

- data has been obtained on the penetrability of formaldehyde gas through various barriers containing biological indicator strips under cold sterilization conditions.

- laboratory studies have indicated that the inactivation of spores embedded in RTV-3140 potting compound occurs at proportionally slower rates as the relative humidity of preconditioning and curing atmospheres increases.

- exposure of mated surfaces of aluminum and stainless steel tubing connectors to 0.1 and 1% paraformaldehyde-trichloroethylene spray resulted in a 1-2 log reduction of inoculated spores over a 168 hour exposure period.

- similar exposures of occluded surfaces or threads of aluminum or stainless steel tubing connectors resulted in total or near total kill of spores in 48 hours with 1% paraformaldehyde-trichloroethylene spray or 72 hours with 0.1% paraformaldehyde-trichloroethylene spray.

- through repeated field trials exploring sterilization parameters, the feasibility of sterilizing a spacecraft mock-up, the Technology Feasibility Spacecraft (TFS), was demonstrated. The TFS, a 20-foot diameter mock-up, was housed in a 24-foot diameter gas tight chamber of approximately 158 m^3.

- an effective portable gas generator system (less than 25 lbs.) with associated filters and blowers was designed and fabricated for sterilizing the TFS.

- a filtration system and highly efficient chemical neutralization system for formaldehyde gas were developed.

- a 50 cubic foot laboratory prototype chamber, equipped with a Company fabricated generator and filtration system, was used for parametric studies on formaldehyde sterilization. Concentrations of gas from 1 to 18 mg/liter were studied.

- the influence of preconditioning spores at various relative humidity levels prior to exposure to 1.0 mg/l of formaldehyde gas was more noticeable at 25C exposure temperature than at 40C.

- studies on the effect of exposure humidity on formaldehyde activity showed that spores on external surfaces were inactivated more rapidly at higher exposure humidities whereas studies using various film

barriers showed that penetration, and hence inactivation of spores, was more rapid at the lower humidity levels.

residual formaldehyde was not detectable on hard surface items after exposure to a gas concentration of 1.0 mg/l and was dissipated within seven days from soft or porous materials after aeration at ambient conditions.

BACKGROUND

In recent years there have been a number of attempts to produce coatings or produce additives that will result in materials with self-sterilizing properties (7, 10). Generally, little or no success in achieving sterility has resulted from the use of non-volatile or low-vapor-pressure chemicals (7). This is primarily because such chemicals usually require high moisture environments for maximum bactericidal activity (3, 6, 8) and, even in this instance, show only limited sporicidal activity. On the other hand, some success has been experienced in the development of self-sterilizing materials that incorporate volatile-type disinfectants (7). Nevertheless, the usefulness of a volatile germicide is maximized if volatility is very low at ambient temperatures yet significant at elevated temperatures below 100C.

Studies conducted at Becton, Dickinson and Company laboratories prior to initiation of the contract studies demonstrated the potential usefulness to spacecraft sterilization problems of organic chemicals containing a volatile chemical germicide. Specifically, it appeared feasible and practical that sterilant mixtures could be developed that would be relatively inactive at ambient conditions but when placed at moderately elevated temperatures would evolve sufficient amounts of sterilant to inactivate large numbers of bacterial spores.

The rationale for our studies was based on the knowledge that various organic resins and polymers, when exposed to elevated temperature, will release potentially sterilizing quantities of gaseous formaldehyde along with the required amount of moisture. The active formaldehyde evolves from the organic resin or polymer in such a manner that the rate of release,

and therefore the sterilization process, is a function of time and temperature. The amount of sterilant gas and moisture released is extremely small and contaminating microorganisms are theoretically subjected to in situ sterilization without untoward effects on the electronic parts, mated surfaces, or surrounding areas.

The chemical compounds under investigation possessed the common property of liberating formaldehyde gas upon elevation of temperature. Included in the studies were representative compounds of the synthetic resins and the polymer, paraformaldehyde. The synthetic resins used are reaction products of melamine, urea and phenol with formaldehyde. The major chemical properties of paraformaldehyde are listed in Table I, Appendix.

Melamine (2,4,6 triamine - 1,3,5 triazine) reacts under alkaline conditions with formaldehyde to give various methylol derivatives (16). Because six reactive sites are available, the hexamethylol melamine compound can be prepared. However, the most commonly used product is the methylated trimethylol melamine (16), the structure of which is depicted in Figure 1, Appendix. Cationic resins can be formed with melamine formaldehyde under acidic conditions (15), which are used to impart wet strength properties to paper and shrink resistance properties to textiles.

The reaction products of urea and formaldehyde are a mixture of mono-methylol urea and di-methylol urea (15). The mono-methylol urea compound, with only one formaldehyde reactive group, is not as effective in imparting stability to cellulosic fabrics as is the di-methylol urea (16), the structure of which is shown in Figure 2, Appendix. Urea formaldehyde is used in the paper and textile industry to provide desired properties to these materials,

either as a precondensate (i.e., mixture of mono-and di-methylol urea) which penetrates the cellulose fibers to form the three-dimensional resin in situ or as a partial condensate which does not penetrate the cellulose fibers but instead forms the three-dimensional resin on the fiber surface (15). Aqueous urea formaldehyde solutions differ from formalin solutions by containing methylol ureas of low molecular weight and water-soluble urea formaldehyde condensates; some free and loosely bound hydrated formaldehyde (methylene glycol) is present (25). Aqueous formaldehyde (formalin) is composed mainly of methylene glycol and various polymeric hydrates (polyoxymethylenes); very little unhydrated monomeric formaldehyde is found (19).

The phenol formaldehyde resins represent many complex products which are produced by reacting formaldehyde with various phenolic compounds (22). The reaction of formaldehyde with the phenols can be catalyzed by both acidic and alkaline conditions thereby resulting in products ranging from simple methylol and methylene derivatives (Figure 3, Appendix) to very complex resins (23). Phenol formaldehyde resins are defined as "mixtures of polymethylene compounds in which phenolic radicals are linked by methylene groups" (24).

Paraformaldehyde is defined as "a mixture of polyoxymethylene glycols containing from about 90 to 99 percent formaldehyde and a balance consisting primarily of free and combined water" (20). It is not a new compound, having been first prepared in 1859 (2), and named paraformaldehyde in 1888 (18). The chemical composition of paraformaldehyde is shown in Figure 4, Appendix, where "n" may represent from 8 to 100 formaldehyde units.

Because of the relative insolubility of paraformaldehyde in acetone, a property not exhibited by low molecular weight polyoxymethylene glycols, the majority of the polymeric forms of paraformaldehyde contain more than 12 formaldehyde units (21). In appearance, paraformaldehyde is a colorless solid, which may be flaky, granular, or a fine powder. At ambient temperatures, paraformaldehyde slowly vaporizes releasing monomeric formaldehyde gas; probably accompanied by water vapor (11). The rate of depolymerization is a function of heat and availability of polymeric end groups (22), since depolymerization occurs at the hydroxyl end groups as an "unzippering" reaction. Thus, paraformaldehyde composed of high molecular weight polymers will evolve gas more slowly than that composed of low molecular weight polymers, although the depolymerization rate is the same for both. The end result of polymer breakdown is the formation of water from the terminal methylene glycol residue. With improvements in processing, the paraformaldehyde manufactured today has a relatively narrow molecular weight range.

The application of formaldehyde gas to inactivate microorganisms was determined and practiced before the turn of the century. One of the first applications was using formaldehyde as a wet vapor to fumigate sick rooms (14). Later Walker (26) found formaldehyde gas to be effective by the addition of a chemical to liquid formalin to produce the gas. Pernot (12) found success using formaldehyde gas to sterilize surfaces of eggs in incubators by adding potassium permanganate to formalin. In 1939, Nordgren (11) summarized much of the early work concerning the efficacy of formaldehyde gas. With an increased need for area sterilants, vaporized formaldehyde found extensive use as a space and surface sterilant showing effectiveness against bacteria, fungi, viruses, and rickettsia, as well

as insects and other animal life (4). Three important factors associated with the positive action of formaldehyde as a gaseous fumigant during this early period were elevated temperature, high relative humidity, and questionable penetrability (13). These factors concerning the action of formaldehyde were determined from the application of liquid formalin, thereby producing a wet vapor containing formaldehyde gas.

In 1956, Kaitz (9) introduced a unique application of formaldehyde gas with a process that involved the depolymerization of the formaldehyde polymer, paraformaldehyde, to yield dry formaldehyde gas for use in area and surface sterilization. In 1961, Harry (5) confirmed the work of Kaitz by effectively ridding surfaces of poultry houses of bacterial life. Later Vineland Poultry Industry (19) reported many applications for surface sterilization using paraformaldehyde. In 1969, Taylor et al (17) presented a broad spectrum of paraformaldehyde applications for surface sterilization and detoxification using dry formaldehyde gas.

Studies have demonstrated that by using the paraformaldehyde polymer, sterilization can be achieved at ambient relative humidity and temperature with no significant build-up of residuals or obvious damage to materials. Aeration of an area can be achieved in a relatively short time period. In addition, it has been determined that the dry formaldehyde gas can penetrate closed areas and packaged materials. The gas concentration can be controlled to any desired concentration by weighing exact amounts of the paraformaldehyde chemical. This new concept of gaseous formaldehyde sterilization is quite different from the early use of vaporized formaldehyde and has opened new vistas for polymer-resin research in the field of sterilization.

We are convinced, as other investigators are, that numerous advantages accrue with the use of the dry gas as compared to the vaporized formalin. Among these are ease of handling, need for less material, no residues, insignificant adsorption and repolymerization, much better penetration, accurate quantitation, incorporation into carrier materials, and others.

The results of studies reported here have supported our conviction that the use of dry formaldehyde gas can be further extended to the sterilization of spacecraft surfaces, parts, and materials.

METHODS AND MATERIALS

Selection of Candidate Carrier Materials

Studies were initially conducted on the screening and selection of suitable sterilant carrier materials from a number of adhesives, sealants, coatings, and potting compounds. Their suitability for use was determined by several criteria including physical properties (color, odor, etc.), handling properties (viscosity, mixing, etc.), curing properties (bubble formation, curing time, exothermic reactions, moldability, etc.) and post-cure characteristics (handling, ease of grinding or cutting for removal of organisms, etc.). It was essential that the sterilant carriers selected not be highly exothermic and be conveniently handled and manipulated by procedures for embedding and recovering of spores during internal sterilization studies. The potting compound RTV-3140 (Dow Corning) possessed a number of favorable characteristics and was used extensively for exploratory work performed during the contract feasibility study (NASw-1764). Since testing of a variety of compounds was beyond the scope of the initial study, this task was conducted as a part of the present investigation. Candidate carrier materials evaluated included the following:

- Lucite
- Stycast 2850 GT with curing agent (Emmerson & Cuming)
- Polyurethane with curing agent 7139 (Whittaker Corp.)
- Resin C-1 with activator E (Armstrong Prod. Co.)
- Compound 3547 (Chem Seal Corp.)
- Epoxy Resin Adhesive A-Z (Armstrong Prod. Co.)
- Napcofoam Urethane with curing agent (Napco Chem. Co.)
- PR-1535, Parts A and B (Product Research & Chem. Co.)
- PR-1538, Parts A and B (Product Research & Chem. Co.)
- Eccofoam FPH #178 (Emmerson & Cuming)
- Rigidax Flakes
- Aluminum 2014 with adhesive film (Bloomingdale HT 424)
- RTV-118 (General Electric Co.)
- RTV-731 (Dow Corning)

Each of the above materials was prepared according to manufacturer's directions and evaluated for usefulness according to the stated criteria. The majority of compounds tested were rejected on the basis of failure to comply with one or more requirements such as ease of mixing active ingredients and/or additives, ease of manipulating uncured material, no excessive entrapping of bubbles, or ease of recovery of embedded spores by established methods.

In the preparation of lucite, preliminary tests were conducted with several monomer and ground polymer combinations to determine the optimum type and ratio of substances to use. The ground polymers tested were:

- "Quickmount" polymer and monomer kit (Fulton Metallurgical Produces Corp.)
- Transoptic powder 20-3400 (Buehler, Ltd.)
- Methyl methacrylate M-215 (Fisher Scientific Co.)

The monomer used was methyl methacrylate monomer 112 (Dupont Chemical Co.).

Lucite rods were formed according to the method of Angelotti, et al (1). Equal parts of Dupont methyl methacrylate monomer 112 and Buehler transoptic powder 20-3400 were mixed and placed in 20 x 150 mm glass test tubes. Bubbles were removed under vacuum (17 mm mercury) prior to completion of the curing process where the tubes were placed in a 50C water bath for 2 hours. No interference with the polymerization process was noted as a result of the addition of 5% paraformaldehyde (formaldegen) or 9.5% melamine formaldehyde (cymel). Complete dissolution of the sterilant additives did not occur upon incorporation into the mixture and the materials were evenly dispersed in particulate form throughout the clear lucite rods.

Control discs (no additives) and discs containing either 5% paraformaldehyde or 9.5% melamine formaldehyde were used for studies to determine formaldehyde release. Lucite rods were sectioned on a lathe into 1/8" thick discs. The cutting technique produced a semi-polished finish across the diameter of the disc similar to that of the edge which was smoothed by the mold surface. The release of formaldehyde gas from the lucite discs was determined on the basis of weight loss as a function of time and temperature. Biocidal activity resulting from residual sterilant additive was determined by zone inhibition studies using Escherichia coli and Staphylococcus aureus as test species (method discussed on page 18).

RTV-118, RTV-731, and Chem Seal 3547 were prepared in 25 ml volumes and subsequently cured according to the manufacturer's recommendations. Formaldehyde containing additives were mixed into the compounds before pouring into molds consisting of 20 x 150 mm plastic disposable petri dishes.

Napcofoam and Eccofoam were prepared in 50 and 25 ml quantities, respectively. After mixing with catalyst, they were placed in 250 ml plastic disposable beakers which served as molds. The solidified foams were allowed to cure at ambient temperatures for 18 hours (Napcofoam) and 24 hours (Eccofoam) after which they were removed and sliced into 1/4" thick wafers. A cork borer was utilized to remove circular discs of 10 mm diameter from the slices of foam material. Control discs and discs containing sterilant additives were employed in zone inhibition studies according to the procedure previously described.

Verification of Internal Sterility of Sterilant-Carrier Systems

Studies on the inactivation of bacterial spores embedded in potting compound were conducted with B-D certified biological indicators (1×10^5 spores of Bacillus stearothermophilus on filter paper strips).

For verification of internal sterility of potting compounds, biologic indicators were placed within 0.5 mil plastic (Teflon) pouches, sealed, and then embedded in potting compound or carrier material, with or without additive, prior to the curing process (Figure 5, Appendix). The actual method involved the use of 25 ml uncured carrier-sterilant mixtures, one-half of which was placed into petri plates. From three to nine sealed biologic indicators were then placed onto the surface of the uncured carrier-sterilant mixture and the reminder of the 25 ml volume poured over the plastic pouches containing the bacterial spores. The plates were allowed to cure at ambient temperature for 72 hours prior to further evaluation at elevated temperatures. Control studies indicated that sufficient formaldehyde gas was not generated during the curing process to inactivate the biologic indicators. Additional studies where embedded spores were held for 96-144 hours at ambient temperature gave the same results. During subsequent internal sterilization trials, the embedded spores were subjected to various time-temperature exposure periods, after which the strips were removed aseptically and assayed by qualitative or quantitative procedures. Qualitative assays were made by inoculation of spore strips into Trypticase Soy Broth (TSB). The incubation temperature for B. stearothermophilus was 60C for periods of at least four days with daily observation for presence of growth. Positive cultures were examined for possible contamination in order to eliminate false positives.

Quantitative assays were made by introducing spore strips into 18 x 150 mm screw cap test tubes containing glass beads in 10 ml distilled water. The strips were agitated for at least 15 seconds on a Vortex mixer which resulted in complete disintegration of the strips. After a series of ten-fold dilutions, aliquots of each dilution were plated in triplicate in Trypticase Soy Agar (TSA). Colony counts were recorded after incubation at 60C for 48 hours.

Studies on the addition of various chemical sterilants to potting compounds and other carrier substances were conducted with the use of specific amounts of resin or polymer and sterilant carrier. The sterilant-carrier combinations were mixed thoroughly and then allowed to cure as required. In each case, a negligible weight loss occurred during the curing period. The concentrations of the polymer, paraformaldehyde (1.0, 5.0, and 10.0%), and the synthetic resins, melamine formaldehyde (1.9, 9.5, and 19.0%) and urea formaldehyde (2.1, 10.6, and 21.0%) were calculated on a weight/volume basis to insure the availability of equal numbers of formaldehyde molecules in corresponding samples of sterilant-carrier systems. For instance, carriers containing 1.0% paraformaldehyde, 1.9% melamine formaldehyde, and 2.1% urea formaldehyde were tested together.

For zone inhibition tests, using a modification of the antibiotic disc sensitivity procedure, sterilant-carrier discs were placed on TSA plates previously inoculated with 24-hour cultures of test organisms. In most studies, E. coli (gram negative rod) and S. aureus (gram positive coccus) were used as test species. However, certain others were occasionally employed including Bacillus subtilis var. globigii, Serratia marcescens, and Klebsiella pneumoniae. The discs were usually exposed to given time-

temperature intervals prior to their use in zone inhibition tests as a means of determining available residual formaldehyde. Zone inhibition readings were made at 24, 48, and 72 hours after inoculation; zone diameters were measured in millimeters.

The methodology employed in the determination of formaldehyde gas loss from melamine formaldehyde, paraformaldehyde, and urea formaldehyde as a function of time and temperature included series of accurate weight assays using an analytical digital Mettler balance. Appropriate control samples were routinely included in all trials. Individual experimental points were assayed in triplicate and repeated at least twice; 100 mg samples of polymer or resin were used in aluminum weighing pans. The studies on loss of formaldehyde from various carrier-sterilant mixtures were conducted in a similar manner. Results were corrected for any weight loss recorded for control discs.

Spray Application of Sterilant

Experiments were conducted to determine the suitability of trichloroethylene as a liquid carrier for formaldehyde-liberating additives for spray, dip, or brush application. Stainless steel and glass strips were coated with 0.1%, 1.0% and 10.0% concentrations of paraformaldehyde or melamine formaldehyde by dipping and spraying procedures. Dried strips were viewed under a microscope at 10X and 70X magnifications to determine uniformity of distribution of dried sterilant crystals. The most satisfactory results were obtained when strips were laid flat and sprayed with an aerosol generated by a power sprayer unit. A uniform layer of sterilant crystals was observed on the surface of all test strips exposed in this manner. Application of sterilant by a dipping technique resulted in an uneven

distribution of the substance with a heavier concentration of crystals at one end of the strip than at the other.

After establishing the methodology required for spray application of sterilant, subsequent studies were carried out on the sterilization of occluded and mated surfaces of spacecraft tubing connectors by application of 0.1 and 1.0% concentrations of paraformaldehyde in trichloroethylene. The threads (representing occluded surfaces) of stainless steel and aluminum connectors, lubricated or non-lubricated, or mating surfaces of connectors (represented by the beveled end of the connector and the flared end of tubing) were inoculated with approximately 1×10^5 spores of B. stearothermophilus in a 0.01 ml volume. The inoculated assemblies were dried in a laminar flow hood and appropriate nuts torqued to 125 lbs. Previous accurate measurement of a complete assembly indicated that 41.86 cm^2 comprised the total surface area. Thus, rather than attempting to spray the assemblies themselves, in which case reproducibility would be a problem, a piece of aluminum of the same surface area was prepared and used as the carrier of the sterilant spray. Exposure procedures involved the placement of an inoculated assembly along with the sprayed aluminum piece into heat sealed aluminum pouches for exposures ranging from 3-168 hours at 60C. For assessment of viable organisms, the pieces were disassembled and placed into sterile TSB for removal of organisms by insonation. Appropriate dilutions were plated in triplicate in TSA and colonies were counted after 48 hours incubation at 60C.

Dimethyl Sulfoxide Studies

Studies on the effect of dimethyl sulfoxide (DMSO) when used in conjunction with formaldehyde gas were initially conducted on a qualitative basis.

Biologic indicators containing 1 x 10^5 spores of B. stearothermophilus were inoculated with 0.02 ml of undiluted DMSO after which the strips were suspended in VACUTAINERS® in the presence of 1 mg paraformaldehyde. Exposures were carried out at 90C for intervals of 15-90 minutes. Exposed strips were aseptically placed in tubes of TSB for the recovery of viable organisms.

Quantitative studies were conducted using a specially-constructed apparatus for injection of DMSO and formaldehyde vapors into an exposure chamber separately or in combination. The all-glass apparatus consisted essentially of DMSO and formaldehyde generators (32 x 200 mm test tubes containing DMSO or paraformaldehyde) and a dessicator jar which served as the exposure chamber. All components were at ambient temperature during testing except the formaldehyde generator which was immersed in a constant temperature water bath at 60C.

Additional quantitative inactivation experiments were conducted using a modified gas generating apparatus. In the modified apparatus, the entire system was initially evacuated to 4 mm Hg following equilibration of the apparatus to 60C in an incubator chamber. Gas vapors of each substance were then introduced into the exposure chamber separately or in combination by means of stopcocks included in the tubing system. Tests were also conducted at atmospheric pressure using the modified apparatus. In all quantitative studies, indicator strips were removed after exposure, disrupted in 9 ml water blanks containing glass beads and plated in triplicate in TSA.

In an effort to promote penetration of formaldehyde gas, DMSO was utilized in several trials on the inactivation of spores on mated surfaces of tubing

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connectors. In each trial, a torqued assembly and piece of aluminum sprayed with 1.0% concentrations of paraformaldehyde in trichloroethylene and comprising 41.86 cm^2 surface area were placed into an aluminum pouch as described previously. A 2 ml volume of DMSO was inoculated onto the surface of a piece of Sensi-disc* paper of the same surface area as the sprayed aluminum piece. After the DMSO had soaked uniformly into the Sensi-disc paper, the paper was placed into the aluminum pouch on the side of the connector opposite that on which the sprayed aluminum piece was placed. Exposure procedures were carried out in hermetically sealed aluminum pouches for exposures of 0-168 hours at 60C.

Preconditioning and Embedding of Spore Strips under Controlled Humidity

Parametric experiments were conducted to determine the influence of relative humidity and cellular water content on the rate of inactivation of bacterial spores in RTV-3140 potting compound containing 0.1% paraformaldehyde. Paper strips were inoculated with 1×10^5 spores of B. stearothermophilus and heat sealed in 0.5 mil Teflon after drying overnight in a laminar flow hood. The sealed strips were subsequently placed at 25C in constant humidity chambers at relative humidity levels of 17, 32, 55, or 75%. After three days of exposure to a given humidity, the spore strips were rapidly embedded in RTV-3140 potting compound with or without paraformaldehyde and returned immediately to their respective constant humidity chambers for curing, which required approximately seven days. Upon completion of the curing period, the embedded spore strips were removed and exposed to 60C in an oven for various time intervals. Following each exposure interval, strips were removed and quantitatively assayed as previously described.

*BBL, Division of BioQuest, Cockeysville, Maryland

Parametric Studies on the Sterilization of Spores on External Surfaces

The studies on the influence of relative humidity on the inactivation of RTV-3140 embedded spores by formaldehyde gas were extended to include a study of the influence of relative humidity and cellular water on the inactivation of spores on external surfaces.

The exposure chamber used in the latter studies was a specially designed stainless steel experimental chamber which permitted precise control of all desired parameters. Basically, the chamber was a horizontal cylinder, 3 feet in diameter by 4 feet in length which was supported 3 feet off the floor on a mild steel supporting framework. One entire end of the chamber could be opened to provide access to the interior. A 2 x 4 foot perforated work surface inside the chamber provided a flat area for placement of test samples. A 4-inch diameter stainless steel pipe extended from the floor of the chamber, near the door, externally to the center of the convex rear wall of the chamber. A 2 horsepower blower placed in-line in this piping permitted circulation of the chamber atmosphere. The chamber atmosphere was drawn through the perforated work surface and into the floor pipe; this pipe reentered the chamber at the rear and a baffle plate forced the atmosphere to the top of the chamber where it was forced down through a perforated ceiling. A hot water heat exchanger was mounted in the piping and all flow was directed through the exchanger which provided heating and cooling of the chamber atmosphere. Humidification in the form of dry steam was introduced directly into the chamber from the source. When the door was bolted closed, a clear view of the interior was provided through two 8-inch diameter glass windows in the door. In addition to precise control of all parameters, the unique feature of the

gas circulating system permitted exposures to be conducted in static or dynamic modes.

Paraformaldehyde was utilized as the source of monomeric formaldehyde gas. The monomeric gas molecules were released by thermal depolymerization at a temperature of 140C. Heating of the powder was accomplished in a specially adapted airtight canister constructed of stainless steel and placed on an electric hot plate. The canister was attached externally to the exposure chamber by flexible plastic tubing wrapped with heating tape. Temperature measurement of the interior of the gas disseminating vessel was provided by means of a copper constantan thermocouple which entered the vessel through the removable cover. A pressure relief valve was also placed on the vessel which permitted gas to be vented into a water drain if necessary.

Following the completion of each exposure, the chamber was purged of formaldehyde gas by three successive evacuations to -25" Hg pressure. Evacuated air was continuously bubbled into a water tank which emptied into a drain pipe. In addition, chamber gas was evacuated by an auxiliary vacuum pump and passed into a filter bed containing a formaldehyde oxidizing-deodorizing material (Purafil) composed of activated alumina impregnated with potassium permanganate. The filter material adsorbed formaldehyde and then oxidized the gas, rendering it harmless and odorless.

Spores of B. subtilis var. niger were used as the biological challenge during these studies. Stainless steel strips (15 x 50 mm) and Whatman #1 filter paper strips (4 x 25 mm) served as carriers for 80% methanol-suspended spores at a concentration of 1×10^6 spores per strip. Prior to exposure,

groups of dry inoculated strips were placed in constant humidity chambers for three to four days at 15, 30, 50, and 70% R.H. Following the conditioning period, the strips were removed and placed in the sterilizer chamber. Gas exposures were conducted at chamber relative humidities of 30, 50 or 70% at a temperature of 25C or 40C. The concentration of formaldehyde in all trials was 1.0 ± 0.1 mg/l as determined by a modified chromotropic acid assay procedure. Exposures at a chamber temperature of 40C were conducted for periods ranging from 30 minutes to 6 hours while those conducted at 25C ranged from 3 to 18 hours. Four strips were quantitatively assayed for survivors for each point using TSA as the recovery medium.

The inoculated and exposed stainless steel strips were assayed by placement in screwcap test tubes (25 x 200 mm) containing 50 ml of 1% peptone-0.5% Tween 80 solution, which were suspended approximately 1 inch from the bottom of a sonicator bath tank (Branson, Model LT-80-6) and subjected to sonication for 12 minutes at 100% power output. Subsequently, 5 ml portions were aseptically pipetted from the test tubes into plastic petri dishes or, if required, initially into dilution blanks of sterile distilled water. Twenty ml of sterile molten (50C) TSA was added to each plate and the contents were mixed by gentle swirling. After solidification of the mixture, all plates were incubated at 35C. Viable spores were enumerated by colony counts after 48 and 72 hours.

Paper strips were placed in screw cap test tubes (1 x 150 mm) containing 10 ml of 1% peptone-0.5% Tween 80 solution and glass beads. The tubes were vigorously agitated on a Vortex "Genie" mixer for 30 seconds. Samples were withdrawn and plated quantitatively for survivors as described above except that 1 ml aliquots were used instead of 5 ml because of the smaller volume of suspending fluid used in the tubes.

Gas Permeability Studies

Corollary studies on the effect of moisture as related to the sterilization of spores enclosed within barriers were also conducted. Spores of B. subtilis var. niger were inoculated on strips (4 x 25 mm) of Whatman #1 filter paper to give a concentration of 1×10^6 spores per strip. The inoculated spore strips were allowed to dry in a laminar flow hood after which individual strips were placed in open-end glass tubes (10 x 110 mm). Each end of the glass tubing was then enclosed in 3 mil or 6 mil polyethylene film, cellophane, or cotton plugging. The barrier-enclosed strips were then placed in a constant humidity chamber at 50% R.H. for four to five days before exposure to formaldehyde gas at a concentration of 1.0 ± 0.1 mg/l. The relative humidity in the exposure chamber was 30, 50, or 70% and trials were conducted at temperatures of 25 or 40C. Exposure intervals were 3, 6, and 18 hours for 25C trials and 1/2, 3 and 6 hours for 40C trials. All paper strips were assayed for survivors by the method described previously. Four strips were assayed in triplicate for each point.

Formaldehyde Residual Studies

Studies to determine the quantity of adsorbed formaldehyde gas remaining on the surface of exposed materials were conducted after the completion of a number of different cycles in which cycle parameters were varied. The materials exposed consisted of strips (15 x 50 mm) of glass, stainless steel, and plastic, in addition to cotton gauze patches (0.3 gm) and 1 inch squares of Whatman #1 filter paper. In certain other gas cycles, conducted at gas concentrations greater than 1.0 mg/l, strips of polystyrene, silicone and latex rubber (1 gm) were exposed and tested for formaldehyde residue after completion of the cycle and after various holding times at ambient

conditions. Following removal from the exposure chamber, each item was placed in 20 ml distilled, deionized water contained in a 25 x 100 mm screw cap test tube. The tubes were heated at 100C for 15 minutes in a hot air oven after which the amount of formaldehyde residue was assayed by the modified chromotropic acid procedure.

Formaldehyde Gas Sterilization of Spacecraft Mock-up Surfaces (MSFC Field Trials)

The objective of the field trials conducted at Marshall Space Flight Center was to investigate under simulated full scale conditions the feasibility of using dry formaldehyde gas for the cold sterilization of external spacecraft surfaces. The field work was supported by corollary laboratory studies on the effect of various parameters on formaldehyde sterilization of spores on external surfaces.

Sterilization Chamber

Before initiating gas sterilization studies, it was necessary to make numerous modifications to the MSFC chamber for conversion from a dry heat to a gas sterilization facility. Major alterations involved the complete sealing of the chamber to render it gas tight and the installation of ducts for dissemination and circulation of formaldehyde gas throughout the test chamber and the Technology Feasibility Spacecraft (TFS) housed within the chamber. The latter modification resulted in a complete bypass of the original air circulation system used in dry heat studies and a concomitant reduction in the total volume of the chamber duct system. A diagrammatic view of the original chamber duct system as used in dry heat studies is shown in Figure 1. The modified duct system is diagrammed in Figure 2 and shows that the large duct leading into the top conical portion and associated in-line blower

and large lower exhaust duct were not included in the modified gas circulation system. The smaller of the horizontal ducts of the original system, which joined the chamber at the top of the vertical portion of the wall, was retained in the modified system as the main gas inlet duct. As shown in Figure 2, this duct was extended into the chamber where it split into two branches, one of which led to the top of the chamber and directed gas flow over the TFS and the other duct which led directly into the TFS. Figure 2 also shows a short length of duct work leading from the lower chamber wall to the gas disseminator vessel. Formaldehyde gas was recirculated through the system during and after thermal depolymerization of paraformaldehyde powder within the disseminator vessel, which continued to serve as part of the external gas recirculation system. A second port and short piece of duct work was placed at the base of the wall beside the port shown in Figure 2 and was utilized as an auxiliary exhaust duct during the removal of gas from the chamber. Flexible 20.32 cm diameter hose was used between the chamber exhaust duct and disseminator vessel, between disseminator vessel and blower, and between blower and gas inlet duct leading into the top of the chamber. A 15.24 cm diameter hose led from the auxiliary exhaust duct at the chamber base and connected to an externally located formaldehyde extraction filtration system. Other modifications included the installation of four ports in the chamber wall, three of which permitted gas samples to be withdrawn from the chamber interior and the other which permitted gas samples to be withdrawn from the interior of the TFS (Figure 3). Other access ports were installed in the chamber wall for passage of electrical cords to the interior of the chamber and for introduction of neutralizing gas. Biological filters

were fabricated and placed in-line in the gas inlet and both gas exhaust ducts.

Formaldehyde Disseminator

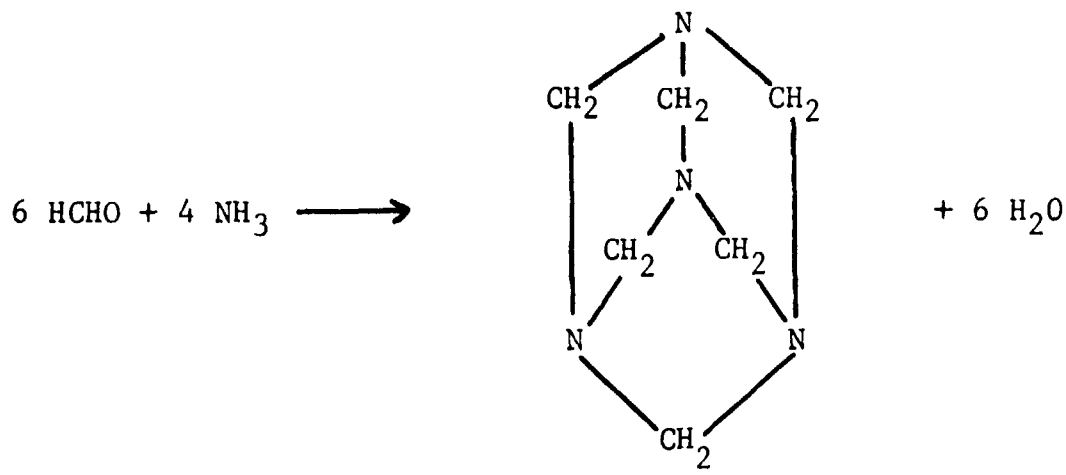
The unit employed for the generation and dissemination of formaldehyde gas was comprised of a heavy duty commercial pressure cooker modified as required by BDRC engineering personnel. The position of the disseminator in the gas circulation system is shown in Figure 2.

Two 5.08 cm ports on opposite sides were placed in the wall of the disseminator for connection to horn-shaped aluminum pipes which flared from a 5.08 cm diameter to a 20.32 cm diameter. The aluminum pipes were attached on each side of the disseminator vessel to the 20.32 cm flexible hoses mentioned previously. A blower unit of the "squirrel cage" type was inserted in-line between the disseminator and the chamber intake port (see Figure 2) and served to recirculate the gas through the chamber and the external gas ducts. In addition to the gas ports, two gas tight Plexiglas viewing ports were placed on opposite sides of the disseminator. These permitted visual examination of the paraformaldehyde powder sublimation process during stages of gas dissemination from the vessel. A heavy duty electric hot plate operated at 350-450 F during the gas generation period provided the source of heat for the gas disseminating system.

Exhaust System

After completion of the exposure cycle, the formaldehyde gas was removed from the chamber and TFS either by internal neutralization and exhaust through chemical filters or by exhaust through the external

chemical filters alone. In those cycles wher formaldehyde was neutralized ammonia gas served as the neutralizing agent by reaction with the formaldehyde to form the neutral product, hexamethylenetetramine, as shown below:



In Trials 3 and 4, ammonium carbonate was heated to obtain ammonia gas. In Trials 5 through 8, a cylinder of pure ammonia gas was utilized as the source of neutralizer. Calculations were made to determine the amount of powder or gas to provide neutralization based on a molar ratio of six formaldehyde molecules to four ammonia molecules. Following the contact period between neutralizer and gas, the chamber door was opened and the exhaust phase initiated. Figure 4 shows the concentrations of formaldehyde during exposure and exhaust after neutralization with ammonia. Both the external blower system and room air fans were used to clear the chamber of neutralized gas and any excess ammonia present. The external exhaust system consisted of in-line blowers connected to filter housings containing the chemical adsorbent, Purafil. The external exhaust system is shown diagrammatically in Figure 3. The hose which connected to the gas inlet duct of the chamber during the gas dissemination and exposure phases was adapted to the filter unit to become part of the exhaust system. Purafil (Borg-Warner Corp., Marbon Chemical Division, Washington, West Virginia), is a solid

odoroxidant comprised of actuated alumina (Al_2O_3) impregnated with potassium permanganate ($KMnO_4$). It is produced in the form of pellets which both adsorb and absorb formaldehyde gas and by oxidation with potassium permanganate chemically destroy the gas until the ambient air contains less than 1 ppm. Figure 5 shows a detailed diagram of the filter housing used for the Purafil bed.

Upon completion of the trials in which formaldehyde gas was exhausted without prior neutralization, the chamber door remained closed and the room air fans were not used during the exhaust period since all of the gas passed through the Purafil filter beds for adsorption. During the exhaust cycle, chamber make-up air was supplied from room air passing through the FG-50 filter in the gas inlet port of the chamber. Figure 6 shows the concentrations of formaldehyde gas during exposure and exhaust without prior neutralization.

Biological Filters

The absolute biological filters used in the chamber were fabricated by BDRC personnel and consisted of 20.32 cm diameter aluminum filter bed housings containing a fiberglass filter medium (FG-50) of 99.96% efficiency. The filter beds were made of two layers of 1.27 cm thick filter media and were placed in-line in the gas inlet and exhaust ports of the chamber.

Biological Sterility Indicators

System sterilization was determined by use of B. subtilis var. niger spores on Whatman #1 filter paper strips (4 x 25 mm) which were placed throughout the chamber and TFS. This organism was chosen for

use because of its resistivity to chemical gases and other sterilization processes. Fifty locations, shown diagrammatically in Figures 7-9 were selected for spore indicator placement and included the interior and exterior surfaces of the TFS and the interior chamber surface. At each location, a set of four spore strips containing 10^4, 10^5, 10^6, or 10^7 spore concentrations in individual paper envelopes was placed. The total spore concentration for all 50 locations was approximately 5.55×10^8. Following the completion of the exposure cycle, strips were removed and placed individually in test tubes containing 15-20 ml TSB. Incubation was conducted at 35C for 21 days before recording final results of the number of positive and negative cultures.

Gas Penetrability Tests

Spore strips were enclosed within different types of barrier materials and included in each of the cold sterilization cycles conducted at MSFC. These provided a means of measuring the penetrability of dry formaldehyde gas under the parameters established in each individual cycle. The types of materials used as gas barriers included polyethylene (2 through 6 mil thicknesses), latex, cellophane-mylar film, glassine paper, screwcapped polystyrene flasks, and cloth covered urethane foam. In most tests, sets of 10^4 through 10^7 spores of B. subtilis var. niger on filter paper strips were utilized. Each individual concentration of spores was individually wrapped and sealed or otherwise placed within the specific barrier material. The barrier-enclosed spore strips were taped on the legs of the TFS prior to initiation of each cycle. Following completion of the gas exposures, the barriers were removed and strips were transferred to test tubes containing 15-20 ml TSB. Incubation was carried out at 35C for 21 days before recording positive or negative growth in the tubes.

EXPERIMENTAL RESULTS

Weight Loss-Zone Inhibition Studies

In studies on the kinetics of formaldehyde gas evolution from lucite conducted at 60C, a 7.6% loss of formaldehyde occurred within 96 hours from 5% paraformaldehyde (PF)-lucite discs while only 5.2% loss was observed from 9.5% melamine formaldehyde (MF)-lucite discs (Figure 10). In zone inhibition tests (Figure 11) conducted with the 17 mm heated discs, slight inhibition of E. coli by the 5% PF discs was observed after each of the exposure intervals through 4 hours. S. aureus was strongly inhibited in the same test. Both test organisms were inhibited throughout a 96-hour period by the lucite-MF discs although biocidal activity decreased progressively with increased exposure time due to loss of formaldehyde from the discs. At 24 hours, zones of 22 mm were observed on plates inoculated with E. coli which decreased to 18 mm after 96 hours exposure. On plates containing S. aureus, 24-hour zones of 43 mm were observed which also decreased to 18 mm after 96 hours exposure (Figure 12).

In weight loss-zone inhibition studies conducted at 90C, 5% PF-lucite discs lost 9.1% formaldehyde after 6 hours exposure while exhibiting very little or no inhibitory activity towards either test organism. A 4.5% weight loss was recorded for the 9.5% lucite-MF discs after 6 hours at 90C and the lucite-MF discs also exhibited good biocidal activity against S. aureus throughout the 6-hour testing period (Figure 13). Zones of 37 mm were observed after 1 hour of heating and decreased slightly to 33 mm after 6 hours heating. E. coli was inhibited very slightly after each of the first 4-hour exposures with no inhibition by discs heated for longer periods. After 96 hours exposure a 19.9% weight loss resulted with no

apparent inactivation of either species by the discs (Figure 14). During the 96-hour exposure periods at 90C, biocidal activity was observed only on plates streaked with S. aureus. After 24 hours, zones of 29 mm were observed which decreased to 19 mm at 48 hours. No biocidal activity was evident after 72 or 96 hours heating and no inhibition of the growth of E. coli was observed after any of the 24 to 96-hour exposure periods (Figure 15).

When lucite-sterilant discs containing 5% PF were exposed to 125C, the loss of monomeric formaldehyde from the discs was very erratic with no definite pattern of release observed during short exposure periods of 1 to 6 hours. Weight losses ranged from 10.7 to 20.4% after 1 hour to 19.8 to 58.0% after 6 hours heating. In comparison, the results obtained after exposure of lucite-9.5% MF discs were very consistent in all tests ranging from 4.2-5.7% after 1 hour to 9.7-10.8% weight loss after 6 hours heating (Figure 16). In the short term exposures, neither the 5% PF nor 9.5% MF discs displayed any biocidal activity against E. coli after any of the exposure intervals. S. aureus was slightly inhibited by discs containing 9.5% MF with zones of 20 and 19 mm observed after 1 and 2 hours heating. No inhibitory activity was observed in the presence of discs heated for 3-6 hours at 125C (Figure 17). After 96 hours exposure to 125C the release of formaldehyde from lucite-5% PF discs was close to maximum at 91.2% loss. The 9.5% lucite-MF discs also exhibited a maximal release with a loss of 14.2% observed. In both instances, the plotted curves began to level off at these points indicating that very little or no additional release of gaseous formaldehyde would have occurred (Figure 18). No biocidal activity of either type of disc was observed against E. coli or S. aureus after the 24 to 96-hour exposure periods.

During inhibition tests conducted with E. coli using non-heat treated discs, zones of 21.3 and 38 mm were observed with discs containing 5 and 9.5% PF, respectively. Zones produced on plates containing S. aureus measured 38 and 34.7 mm, respectively.

Experiments were conducted to determine the loss of weight by plain lucite discs and lucite discs containing 5% PF or 9.5% MF. After 24 hours at 45C, it was found that weight losses of 1.2, 7.0, and 20.3% occurred in control discs, 5% PF discs, and 9.5% MF discs, respectively. The latter two figures revealed a substantial loss of sterilant during curing of the mixtures.

Similar weight loss-zone inhibition studies were conducted with the potting compounds, RTV-118 and RTV-731 containing PF or urea formaldehyde (UF). Tests in which 5% PF was added to each compound indicated that a slightly greater rate of release of formaldehyde occurred from RTV-731. After 6 hours exposure to 60C, approximately 5.2% of the gas had been released from RTV-731 compared to a 4.3% loss from RTV-118 (Figure 19). The results of zone inhibition studies also indicated a consistent and relatively uniform release of formaldehyde from each potting compound-sterilant system throughout the 6-hour observation period. On plates streaked with E. coli, sterilant from the RTV-118 system produced zones of approximately 17.5 to 21 mm diameter. On S. aureus plates, zones of inhibition ranging from 38-44 mm were observed (Figure 20). Relatively uniform results were also observed when the RTV-731 5% PF discs were placed on plates streaked with the two test organisms. Those containing S. aureus showed zones of 46-51 mm while those containing E. coli ranged

from 17-23 mm diameter (Figure 21). The RTV-731 discs produced a slightly greater biocidal effect than the RTV-118 discs against both test organisms. As observed in other tests, S. aureus was more sensitive to formaldehyde than was E. coli.

Studies in which 10.6% UF was added to samples of RTV-118 and RTV-3140 revealed that gas molecules were released more rapidly from the RTV-118 matrix than from RTV-3140. After 6 hours exposure to 60C, RTV-118 sterilant discs showed a weight loss of 5.4% in contrast to a loss of only 2.2% formaldehyde from RTV-3140 sterilant discs (Figure 22). However, RTV-3140 discs produced larger zones of inhibition (Figure 23) than those of RTV-118 (Figure 24). with both test organisms. RTV-118 discs produced zones of 15-17 and 23-31 mm diameter on plates inoculated with E. coli and S. aureus; whereas, RTV-3140 produced zones of 18-23 and 30-36 mm against the same organisms throughout the testing period of 6 hours.

In weight loss studies (Figure 25) conducted with discs of Chem Seal 3547 at 60C, formaldehyde was released in greater quantities from discs containing 5% PF than from those containing 9.5% MF or 10.6% UF. With the exception of a rapid initial release during the first day of the 14-day exposure period, the rate of loss from the latter two systems was reduced but constant. After 14 days the PF discs lost approximately 47% of their initial weight while comparable losses of 4.3 and 4.8% were recorded for the MF and UF discs. The greater loss of formaldehyde during the testing period by the PF discs was reflected in the decrease in size of inhibition zones produced on plates inoculated with S. aureus. Zones produced by discs of MF and UF were of nearly constant diameter throughout the 14-day observation period (Figure 26).

Discs of Napcofoam containing 5% PF were prepared according to a modified curing cycle and observed for biocidal activity at 37C on plates inoculated with E. coli and S. aureus. Limited inhibition resulted after 24 hours incubation with zones of 11 and 21 mm observed against the two organisms, respectively. No inhibitory effects were detected when sterilant discs, prepared according to the manufacturer's specifications, were used in biocidal tests.

Tests conducted using discs of Eccofoam containing 5% PF were indicative of greater biocidal activity against both test organisms as compared to Napcofoam. Zones of 17 mm diameter were produced on plates streaked with E. coli while larger zones of 32 mm were observed on plates inoculated with S. aureus. In tests with Eccofoam discs containing 10.6% UF, biocidal activity was less than either Napcofoam or Eccofoam discs containing 5% PF. Zones of 10 and 12 mm were recorded for E. coli and S. aureus, respectively, with the use of UF.

Comparative biocidal studies (Table I) were conducted with RTV-3140 containing various concentrations of PF (1, 5, and 10%), MF (1.9, 9.5, and 19%) or UF (2.1, 10.6, and 21%). Plates streaked with each of five different test organisms were used. As observed in other tests, Gram positive species (B. subtilis var. niger and S. aureus) were inhibited to the greatest degree while E. coli was least affected. Increases in concentrations of each of the additives resulted in corresponding increases in inhibitory effects.

Table II represents a summary comparison of the effectiveness of the polymer, paraformaldehyde, and the synthetic resins, melamine formaldehyde and urea

formaldehyde in five different electronic materials. The RTV-3140 paraformaldehyde system displayed the greatest degree of biocidal activity against S. aureus whereas E. coli was most effectively inhibited in the presence of the RTV-3140 UF system.

Quantitative Inactivation of Embedded Spores

Quantitative studies were conducted at ambient temperatures using 1×10^5 B. stearothermophilus spores embedded in RTV-3140 containing 1% PF. Without exception, the data obtained revealed an initial plateau period of 4-6 days during which no loss of viability occurred. Subsequently, exponential inactivation took place with a D-value of approximately 60 hours (Figure 27). In comparison, no reduction in spore population was observed in RTV-3140 containing 1.9% MF (1% available CH_2O) or 2.1% UF (1% available CH_2O) during the same curing period. Moreover, no significant reduction in spore population was observed upon exposure of the latter two compounds to 60C for time periods up to 6 hours. Extension of the 60C exposure revealed that internal sterilization of the potting compound was achieved after 24 hours with 1.9% UF, whereas 2.6×10^2 spores were recovered from the RTV-3140 MF system after 48 hours exposure (Figure 28). No loss of viability was observed in control samples during the same time-temperature exposure. These results indicated significant differences in the rate of release of formaldehyde from the three compounds despite the fact that all contained equivalent amounts of available formaldehyde residue. Further studies conducted with 0.1% PF in RTV-3140 at 60C revealed complete inactivation of 1×10^5 B. stearothermophilus spores within 24 hours exposure (Figure 29).

Minimal inactivation (<25%) resulted after a two-week curing period at ambient temperatures when 1 x 10^5 B. stearothermophilus spores were embedded in Chem Seal 3547 containing 1% PF (Figure 30). By comparison, similar studies with RTV-3140 containing 1% PF showed at least a 99% reduction in viable spores during the same curing period (Figure 27). Additional studies were conducted at 60C on the inactivation of 1 x 10^5 spores of B. stearothermophilus embedded in Chem Seal 3547 containing various sterilant additives (Figure 31). The results of these tests revealed complete inactivation of spores in mixtures containing 1% PF after 24 hours exposure, while samples containing 2.1% UF were completely inactivated after 48 hours. Less than 35% inactivation was observed in samples of Chem Seal containing 1.9% MF after 48 hours. These results are in agreement with those of other experiments in which PF was the most effective of the three sterilants with UF and MF following in order.

Sterilization of Occluded Areas of Spacecraft Components

The threads of lubricated and plain stainless steel connectors were inoculated with 1 x 10^5 spores of B. stearothermophilus and exposed to 1% PF for various time intervals at 60C. Results indicated that a 3-log decrease in viable spores occurred within 12 hours on the threads of unlubricated connectors. In subsequent tests, a 4-log inactivation of spores on plain stainless steel connectors was achieved after 48 hours exposure to 1% PF (Figure 32). In tests using lubricated connectors, less inactivation (about a 2-log decline) was observed. Control pieces showed no loss of viable spores throughout the testing period.

In studies using 0.1% PF, a longer exposure period was required to achieve results comparable to those in which 1% PF was used (Figure 33). A 5-log

reduction of viable spores inoculated on the innermost thread of stainless steel connectors was achieved after 72 hours exposure to the reduced concentration of polymer. After 48 hours exposure, a decline of approximately 4 1/2 logs was observed.

When similar studies were conducted using aluminum connectors, a slightly faster rate of inactivation was observed during exposure to 1% PF at 60C. A 3-log decline was observed after 18 hours exposure followed by total inactivation after 24 hours. When the concentration of PF was lowered to 0.1% in subsequent studies. a reduced rate of kill was observed when compared to studies in which 1.0% PF was used. Survivors were present at a concentration of 1.2×10^1 after 24 hours exposure. Thus, in the case of both the stainless steel and aluminum connectors, a reduction in concent.ation of PF from 1.0% to 0.1% caused a corresponding decrease in the initial rate of spore inactivation during the first 24-48 hours of exposure. With stainless steel connectors, effects due to concentration differences were not clearly apparent after approximately 48 hours exposure. Tables III and IV summarize the results obtained in studies on occluded surfaces of stainless steel and aluminum connectors.

Less inactivation resulted from the exposure of B. stearothermophilus spores on mated than on occluded surfaces. Studies were conducted on mated stainless steel surfaces using 1% PF in which an overall reduction of approximately 1 log was observed after 192 hours (eight days) exposure at 60C (Figure 34). The data from trials conducted using a 0.1% PF-trichloroethylene spray indicated an average reduction of nearly 2 logs after 144 hours (Figure 35). Table V is a summary of the results obtained in studies with mated surfaces of stainless steel connectors.

The exposure of mated surfaces of aluminum (blue anodized type) connectors to 1% PF-trichloroethylene spray resulted in a 1 1/2 log decrease in viable spores after 168 hours (seven days) exposure at 60C. DMSO was not effective in enhancing gas penetration, and hence spore inactivation, in studies on sterilization of mated surfaces of stainless steel connectors.

Thus, in all instances, less inactivation occurred when spores on mated surfaces were exposed to PF-trichloroethylene spray than when occluded surfaces were exposed to the same conditions.

Effect of Moisture on the Inactivation of Embedded Spores

Established differences in relative humidity during preconditioning and subsequent embedding of spores in RTV-3140 potting compound resulted in distinctly different rates of inactivation of spores during 60C exposures. Data analysis showed that the time required for sterility increased with increasing relative humidity, perhaps due to greater uptake of available moisture by the potting compound and spores during curing. The average sterilizing exposures were as follows: 17% R.H. (4 hours 30 minutes), 32% R.H. (5 hours 10 minutes), 55% R.H. (7 hours 10 minutes), 75% R.H. (8 hours). These results are expressed in Figure 36 where a linear relationship between relative humidity and exposure time required for sterility at 60C is indicated.

Effect of Moisture on the Inactivation of Spores on External Surfaces

Experiments on the effect of moisture on embedded spores were extended to include the influence of moisture on the inactivation of spores on external surfaces. The data obtained from these studies were utilized to construct thermochemical death time (TCDT) curves by the method of

linear regression. The decimal reduction ("D") value was obtained from the slope of each TCDT curve and represented the time in minutes required to reduce the spore population by 90% of its concentration under the conditions of the experiment

Exposures were conducted at 40C and 25C utilizing spores of B. subtilis var. niger inoculated on filter paper or stainless steel strips.

Inactivation Trials Conducted at 40C

Figures 37 through 39 are TCDT curves showing the inactivation rates of spores on filter paper strips exposed to 1.0 mg/l formaldehyde gas in the presence of 30, 50, or 70% R.H., respectively. Prior to placement in the sterilizing chamber, individual groups of spores, as indicated on the figures, were preconditioned in atmospheres of 15, 30, 50, or 70% R.H. Thus, for example, a typical exposure (such as illustrated by Figure 37) would have included four groups of spores, each of which had been preconditioned at 15, 30, 50 or 70% R.H. and subsequently exposed simultaneously to a concentration of 1.0 mg/l formaldehyde gas in an atmosphere of 30% R.H. In subsequent trials, similarly preconditioned groups of spores were exposed to 1.0 mg/l of formaldehyde in an atmosphere of 50 or 70% R.H. Thus, in any one trial, the only variable was the preconditioning relative humidity level employed prior to exposure of the spores to a given set of cycle parameters.

Data indicated that the rate of spore inactivation increased with increasing exposure humidity and that the rates of kill were influenced more by exposure humidity than by the relative humidity at which the spores were preconditioned. Table VI lists the D-values for each of the trials depicted in Figures 37-39 and shows that large differences in spore inactivation rates due to the preconditioning moisture levels were not observed at any of the exposure

humidity levels tested. However, small increases in death rates at each exposure humidity did occur as a result of increases in preconditioning relative humidity levels from 15% through 70%.

When stainless steel strips were employed as the substrate, the TCDT curves shown in Figures 40-42 and D-values shown in Table VII were obtained. It was evident that the rate of spore destruction was influenced by the relative humidity established during gas exposures in a manner similar to that observed when paper strips were utilized as spore carriers. Significant differences in rate of kill due to variations in preconditioning relative humidities were not apparent.

Inactivation Trials Conducted at 25C

A similar series of experiments were conducted with spores on filter paper and stainless steel strips at 25C.

Figures 43-45 are TCDT curves indicating the rates of spore inactivation of B. subtilis var. niger spores on paper at 25C. Table VIII lists the D-values calculated for each of the trials depicted by the curves in the Figures. As in trials conducted at 40C, the chamber humidity established during exposure cycles was found to be very influential with respect to rate of spore inactivation. This was most evident when exposure R.H. was increased from 30 to 50% and less when proceeding from 50 to 70% exposures.

With respect to the influence of preconditioning moisture levels, it was found that at 30% and 50% exposure chamber humidities the rate of spore kill increased regularly with spores preconditioned at 15% R.H. through those preconditioned at 70% R.H.

Overall, varying preconditioning relative humidity did not exert as clear an influence at 25C on rate of kill of spores on stainless steel as in

trials conducted with spores on paper strips. However, some evidence of rate increase due to increased preconditioning humidity from 15% to 70% was observed during the exposures conducted at a chamber relative humidity of 30%. The results of the trials using stainless steel strips are shown in Figures 46-48 and in Table IX.

Thus, both exposure relative humidity and the preconditioning humidity were found to exert a significant influence on the rate of spore inactivation by formaldehyde at a 25C exposure temperature. These results were slightly different from those obtained in 40C trials where preconditioning relative humidity was found to exert less of an influence on rate of kill than exposure chamber humidity. Apparently, the higher exposure temperature of 40C masked the influence of preconditioning relative humidity to a greater degree than did the 25C exposure temperature.

It is conceivable that at higher exposure temperatures or gas concentrations in excess of 1.0 mg/l, the influence of preconditioning humidity would be completely masked and therefore irrelevant in the design of a formaldehyde cycle for sterilization of exposed surfaces. However, on the basis of these results consideration must be given to the relative humidity of the exposure chamber. This consideration may in turn be influenced depending on whether the gas is expected to act upon exposed surface contaminants or must penetrate materials containing microorganisms.

Effect of Relative Humidity and Temperature on Penetration of Formaldehyde Through Barriers

It was found that the permeability of formaldehyde gas decreased at 25 and 40C as the relative humidity in the exposure chamber increased. At both 25 and 40C, penetrability and hence inactivation of the initial 10^6 spore population was generally less at 70% R.H. than at 50% or 30%,

respectively. These data are expressed as D-values in Table X and as TCDT curves in Figures 49 and 50. It was quite interesting to note that penetrability of the gas was generally better at 25C than at 40C when comparable exposure R.H. levels were compared. For example, the rate of spore inactivation at 25C and 50% R.H. was greater than at 40C and 50% R.H. The reason for this occurrence may have been due to differences in the actual amount of moisture present in each exposure condition. Table XI shows the amount of water vapor in air for each temperature in terms of mg/l. At any given relative humidity, the amount of moisture present in air varied with temperature and less than half as much was present at 25C as at 40C. Apparently, the formaldehyde gas molecules were more readily bound at the surfaces of the film barriers, and in the cotton to some extent, at 40C than at 25C. Thus, the gas penetrated the barriers at a given relative humidity more efficiently at 25C resulting in a faster rate of kill of enclosed spores. As indicated by the data of Table X, the rate of spore inactivation at 30% R.H. and 25C was accelerated to the extent that no survivors were present at the time of the initial sample (3 hours exposure). Since 10^6 spores were exposed, it is assumed that D-values of 50 or less prevailed during these trials as compared to a D-value of 88 for exposure at 40C and 30% R.H. A D-value of 50 or less would be expected in comparison with the results of other tests.

The data may also be examined from the aspect of absolute rather than relative humidity to determine whether in the presence of equivalent moisture concentration, gas molecules vary in rate of penetration with variation in exposure temperature. At 25C and 70% R.H. approximately 14.1 mg/l water vapor were present whereas at 40C and 30% R.H. 15.36 mg/l water vapor were present. Since the difference in moisture levels was insignificant, the moisture factor could be regarded as a constant

and observed differences compared with respect to temperature. Accordingly, it was noted that the rate of inactivation at 40C was greater in each instance except in those involving cotton as the barrier indicating that in the presence of equivalent moisture levels, temperature can also exert an influence on penetration of barrier materials.

Formaldehyde Residual Studies

A number of trials were conducted to determine whether residual formaldehyde could be detected on various types of materials after gaseous exposure as a function of varying parameters.

Assays for the detection of residual formaldehyde on test pieces were conducted immediately after exposure and after holding 7 days under ambient conditions. Immediately after exposure to 1.0 mg/l of gas, hard surface items such as stainless steel, glass, and polypropylene plastic showed no trace of formaldehyde. In comparison, porous materials such as filter paper and cotton contained relatively high concentrations of formaldehyde. Table XII shows the data from assays conducted on various materials exposed to different cycle conditions. Where paper or cotton test pieces were exposed to 1.0 mg/l formaldehyde, no residue was detected after holding under ambient conditions for 7 days. When polystyrene or rubber materials were exposed to increased concentrations of 6-18 mg/l of gas, very little or no residual was detectable after 7 days. These results indicate that residual formaldehyde should not be a significant factor with respect to hard surfaces such as those evaluated in this study. Varying residual levels may occur in or on porous materials such as cloth, paper, and soft rubber. However, with aeration under ambient or elevated temperatures, dissipation of the formaldehyde should occur readily.

Experimental Results of Field Trials on Sterilization of the Technology Feasibility Spacecraft

A total of eight trials were conducted at MSFC on the cold sterilization of external spacecraft surfaces with dry formaldehyde gas. The results of all trials are summarized in Table XIII.

Trial 1 involved the adaptation of equipment to the chamber after initial BDRC directed modification by MSFC personnel. No biological spore strips were placed within the chamber, however, gas was disseminated into the chamber and TFS to test the function of the disseminator, blower, and gas sampling units. Air flow measurements and circulation patterns within the chamber were determined before gas dissemination was initiated. The chemical extraction filters were tested for efficiency following a 12-hour period during which formaldehyde gas was continually recirculated through the chamber and external disseminating system. A number of minor system and equipment problems were resolved during this trial, permitting actual sterilization cycles to commence with Trial 2.

The chamber and TFS were seeded with 50 sets of biological spore strips in Trial 2. Each set of 10^4-10^7 spore strips was placed in a given location (Figures 7 through 9). In addition, spore strips within polyethylene and latex barriers were included (Table XIV) to test penetrability of the gas under the parametric conditions established. The results of this first attempt at sterilization were extremely good considering that the exposure temperature was only 66F. All of the 10^4, 10^5, and 10^6 spore strips were completely inactivated (50 strips of each concentration) and 46/50 spore strips of the 10^7 concentration were inactivated after the 12-hour exposure. All of the strips (10^6 concentration) within the latex barriers were sterile;

however, the gas did not sterilize the spore strips within the 1,2,4, and 6 mil polyethylene barriers. Chemical indicator strips did show that some gas penetrated the 1 mil polyethylene film but in an insufficient amount to sterilize. The results of this initial biological trial indicated the potential feasibility of using formaldehyde gas to sterilize the surfaces of entire spacecraft under cold or ambient sterilization conditions.

Trial 3 conditions included an exposure temperature of 86F and cycle time of 6 hours on the basis of previous excellent results at a lower temperature and longer time. Ammonia gas as released from heated ammonium carbonate powder was used to neutralize the monomeric formaldehyde gas upon completion of the sterilization cycle. Results of this trial showed that 100% sterility was achieved at all spore strip concentrations. Gas penetrability studies using film barriers were not performed in this trial, however, gas residual studies were included to determine the amount of residual formaldehyde detectable on glass, plastic, and stainless steel surfaces. Strips removed immediately after formaldehyde exposure and placed in distilled water tubes for assay showed no trace of residual formaldehdye gas. However, strips removed after the neutralization cycle was completed showed that formaldehyde was bound on the surfaces in the hexamethylenetetramine complex formed by the reaction of formaldehyde and ammonia. Glass adsorbed the greatest amount of hexamethylenetetramine followed respectively by stainless steel and plastic which adsorbed approximately 1/5 and 1/8 as much of the complex as glass.

Trial 4 parametric conditions were essentially the same as those of Trial 3 except that two room air fans were placed within the test chamber to aid

in the dynamic movement of the circulating gas-air mixtures. In addition, pure ammonia gas from a cylinder was used to neutralize the formaldehyde at the completion of the 6-hour exposure cycle instead of ammonia derived from the heating of ammonium carbonate powder. It was calculated that approximately 2.9 cubic feet of gas would be necessary to neutralize a concentration of approximately 1.5 mg/l of formaldehyde gas in the 5560 cubic feet of chamber space. The ammonia was injected directly into the chamber upon completion of the formaldehyde exposure cycle at a rate of 0.15 cubic feet per minute. Upon completion of the ammonia gas injection period, an additional period of 15 minutes was allowed before the chamber door was opened for entrance of personnel. Upon culture of the spore strips, it was found that the results were less successful than in previous trials. As shown in Table XIII, the percent inactivation of spore strips in paper enclosed envelopes ranged from a low of 60% on 10^7 strips to a high of 100% on 10^4 strips with 94% and 88% inactivation of spores on 10^6 and 10^5 levels, respectively. Spores enclosed within permeability barriers (Table XV) were inactivated completely at 10^4 and 10^5 concentrations in 2 mil polyethylene pouches and at 10^4 through 10^6 concentrations in 4 mil polyethylene pouches. Spores enclosed within cloth-covered urethane foam were inactivated at 10^4 and 10^5 levels.

During Trial 5, the room air fans were not utilized for circulation of the air-gas mixture as in the previous cycle and represented the only system modification made. The parameters established were slightly different than in Trial 4 since the gas concentration was 0.2 mg/l higher and the relative humidity averaged 63% or approximately 16% higher than in the previous trial. The temperature and exposure period were the same as employed in Trial 4. Results of this trial are summarized in Table XIII and

show that less success was achieved than in previous trials. Percent inactivation of spores ranged from 38% at the 10^7 level to 92% at the 10^4 level. A greater variety of permeability barriers were included in this trial as shown in Table XVI. The results obtained in the penetrability tests with corresponding barriers used in previous trials were essentially similar. In barrier tests not previously included, it was found that complete inactivation of 10^4 and 10^5 spores occurred within cellophane film; 10^4 spores were inactivated within glassine paper barriers; and both 10^4 and 10^5 spores concentrations were inactivated within cotton barriers and screwcapped polystyrene flasks.

Trial 6 parameters were very similar to those established in Trial 4 except that chamber humidity was controlled throughout the cycle with a dehumidifier and room air fans were not used for circulation of the air-gas mixture during the 6-hour exposure period. Again, the results of sterility tests showed that limited success was achieved as a result of exposure of biological indicators to the cycle conditions established. The percent inactivation of spore concentrations ranged from 14% at the 10^7 level to 70% at the 10^4 level. The results of penetrability tests (Table XVII) were better than in the previous trial and comparable to those of Trial 4. This was basically due to the enhanced penetrability of the gas at the level of relative humidity established during the cycle. The increased inactivation observed in the barrier tests as compared to paper enclosed strips probably resulted from better retention of the gas within the barriers.

The parameters established in Trial 7 were similar to those of Trial 6 except that the exposure cycle was increased to 12 hours in an attempt to achieve

total spore inactivation. In addition to the use of a dehumidifier in the chamber, excellent temperature regulation was achieved in Trial 7 by the use of thermostatically controlled heating lamps placed within the chamber. The dissemination of gas was also semi-automated by the use of a day-night timing device to activate the hot plate used for thermal release of formaldehyde from paraformaldehyde powder. Formaldehyde was neutralized at the completion of the exposure cycle by the injection of ammonia gas into the chamber. As shown in Table XIII, the results of Trial 7 were the least consistent obtained in all trials conducted. The results of barrier tests, shown in Table XVIII were not as successful as from previous trials.

The parameters established in Trial 8 were the same as in the previous trial except that the formaldehyde gas concentration was increased to 2.1 mg/l. In addition to the 50 sets of 10^4-10^7 spore strips normally used, 20 extra 10^4-10^7 sets were placed in the chamber at various locations on the external surface of the TFS and chamber wall. These extra strips were not removed until after the completion of both the formaldehyde gas and the ammonia neutralization cycles. The set of 50 10^4-10^7 spore strips and all barrier enclosed spore strips were removed immediately after exposure to formaldehyde gas. It was the intention of this study to compare the results of sterility tests from both groups of exposed strips in order to determine whether the ammonia gas was interfering with the killing action of formaldehyde by premature neutralization. Results obtained from spores exposed to formaldehyde only or formaldehyde plus ammonia are summarized as Trials 8a and 8b respectively, in Table XIII. The results of permeability tests are summarized in Table XIX. The data indicated that ammonia neutralization of formaldehyde apparently did cause a reduction in spore inactivation. This

was particularly obvious at 10^6 and 10^7 spore concentrations at which reductions in percentage inactivation were 15% and 62%, respectively. Corollary laboratory experiments conducted at the BDRC also indicated that ammonia gas was able to inhibit the effect of formaldehyde gas after exposure of spores to the sterilant. The results from exposure of spore strips to formaldehyde gas only were the most successful achieved since Trials 2 and 3.

DISCUSSION

The initial study conducted by Becton, Dickinson and Company (NASA Contract NASw-1764) on the potential application of formaldehyde-liberating compounds to the internal sterilization of potting compounds indicated that the overall concept was entirely feasible. The study had established the suitability of using RTV-3140 potting compound as a carrier for sterilant additives and an efficient and reproducible procedure for the quantitative assay of internal sterility was developed.

In a continuance of the initial contract, studies were conducted with additional potting compounds and formaldehyde-liberating chemicals. A screening program to select candidate carrier materials and sterilant additives for use in model sterilant-carrier systems was conducted. The carrier materials included flight-approved potting compounds, sealants, adhesives, and covering materials provided by Marshall Space Flight Center.

On the basis of the established criteria, the majority of compounds tested were found unsuitable for use with sterilants except for Chem Seal 3547 and Napcofoam. The properties of Chem Seal 3547 were similar to RTV-3140; however, Napcofoam was a rigid foam and thus differed considerably from the other compounds.

In related studies, Urac 110 (American Cyanamide Co.) and Dantoin 685 (Glyco Chemicals, Inc.) were investigated to determine suitability as sterilant additives. Both chemicals contain formaldehyde residues and liberate formaldehyde gas at elevated temperatures. Loss of weight as a function of elevated temperature of Urac 110 and Dantoin 685 revealed that kinetics of formaldehyde liberation from the two compounds were dissimilar.

At temperatures of 45, 60, 90, and 125C after 24 hours exposure, respective weight losses of approximately 2.0, 2.4, 6.0 and 12.4% were recorded for Urac 110 in comparison to 0.5, 1.0, 32, and 85% weight losses for Dantoin 685 at the same temperatures. The observation that Dantoin 685 liquefied at 90 and 125C was considered as contributory to the overall weight loss since the compound contained only about 20% formaldehyde residue. The incorporation of Urac 110 into RTV-3140 and RTV-118 resulted in a 2-2.5% weight loss at 60C over periods of 1-6 hours with no decrease in biocidal activity as determined by zone inhibition studies. This compound was included as a candidate sterilant in subsequent studies.

Quantitative assay techniques were employed to verify the internal sterility of selected potting compound-sterilant systems. Concurrently, the concentration of sterilant additive (i.e., melamine formaldehyde, urea formaldehyde or paraformaldehyde) was varied in order to establish the relationship between additive concentration and time required to achieve sterility at various experimental temperatures. These studies confirmed earlier findings which indicated that the polymer, paraformaldehyde, was superior to the synthetic resins as a sterilant additive. This compound was thus utilized in most of the subsequent contract studies.

In order to determine whether PF could be applied by spray, brush, or dip procedures, an effective method was developed using sprayed surfaces of metal to determine penetration of the evolved gas into mated and occluded areas of spacecraft parts. Spacecraft tubing connectors served as convenient test pieces since they possessed both occluded (threads) and mated (tubing

and connector surfaces) areas. Test data revealed that the dry gas could effectively penetrate and sterilize occluded areas even at reduced (0.1%) concentrations of paraformaldehyde. Less inactivation of spores inoculated on mated surfaces was achieved, even after extended exposure periods (i.e., using a 0.1% concentration of paraformaldehyde in trichloroethylene, a 2-log reduction was achieved after seven days exposure at 60C). These results were not unexpected since the mated surface area of the tubing connector assembly is intended to be gas tight. However, these results did indicate that dry formaldehyde gas does possess good penetrability for threaded (occluded) and mated areas. The use of DMSO did not enhance penetration or activity of PF in tests using spacecarft tubing connectors. Thus, for very efficient and short term sterilization, PF could be applied directly to occluded and mated surfaces by a spray, brush, or dip procedure.

Experiments on the influence of moisture (% relative humidity) demonstrated the importance of this parameter on the rate of inactivation of spores, especially in the presence of low levels of formaldehyde gas.

Studies conducted on spores embedded in potting compound showed that the rate of internal sterilization was enhanced in the presence of low moisture levels. Spores preconditioned at 75% R.H. and subsequently exposed at the same R.H. during curing of the potting compound in which embedding took place required twice as long for inactivation as spores which were preconditioned and cured at 17% R.H. When the data was plotted from replicate trials conducted with spores exposed to 17, 32, 55, and 75% R.H., the curve obtained showed that exposure time required for sterility increased in a linear manner from the lowest to highest moisture levels investigated. Nevertheless, only 8-hours exposure at 60C and 1% PF was required for inactivation of

spores previously exposed to 75% R.H. Thus, sterilization was performed effectively in the presence of high as well as low moisture levels even though rate differences were detectable.

The differences observed in the rate of kill of spores on surfaces in the presence of the various sterilization chamber exposure humidities were not unexpected. At higher moisture levels, a "formalin effect" is exerted and formaldehyde is more efficient in its killing action. Data indicated that the moisture levels at which spores were conditioned prior to exposure to formaldehyde gas were not as influential as the moisture levels established during exposure. This point was particularly important to elucidate because it was the intent of the contractor to use minimal concentrations of sterilant as a means of reducing or eliminating any potential residual problems. With gaseous sterilants, it is known that one or more interrelated parameters may exert an influence under certain conditions, which may be otherwise masked. Since low concentrations of formaldehyde were employed, it was important to determine how other parameters influenced the efficacy of the gas. Although some differences in inactivation rate were observed with filter paper at 40C as preconditioning humidity levels were increased, these differences disappeared when stainless steel strips were employed as spore carriers. The difference was apparently related to substrate affinity of paper for moisture uptake by absorption as opposed to the amount capable of uptake by adsorption with stainless steel strips. The differences observed, though small in the case of filter paper strips, were nevertheless significant.

Exposure humidity was also very influential in trials conducted at 25C. The effect of preconditioning of spores on filter paper appeared to be

quite influential at 30 and 50% exposure humidity. However, data were somewhat erratic in experiments conducted at a 70% exposure humidity and did not permit an unequivocal conclusion to be made. The only noticeable effect of preconditioning humidity when stainless steel strips were utilized occurred in trials conducted at 30% exposure R.H. As preconditioning humidity levels were increased, the rate of spore inactivation increased until it became relatively constant at 50 and 70% R.H. preconditioning atmospheres. As in trials with paper strips, the data obtained in studies conducted at 70% exposure R.H. were somewhat irregular.

The inactivation rates of spores on filter paper strips and on the surface of stainless steel strips were greatly affected by exposure temperature in addition to the exposure humidity. The D-values of spores exposed at 40C on filter paper strips were less than those for spores exposed on filter paper at 25C indicating a faster rate of inactivation. Similarly, the D-values for spores exposed at 40C on stainless steel strips were less than comparable trials conducted at 25C.

The influence of substrate was also noticeable on the inactivation rates of spores at 25 and 40C. At 25C, the D-values for 30 and 50% exposure R.H. were significantly less for spores on filter paper than for spores on stainless steel strips. However, the differences were less at 70% exposure R.H. Similar results were obtained at 40C and exposure R.H.'s of 30 and 50%. At the 70% exposure R.H., the D-values for spores on stainless steel strips were quite similar to those for spores on filter paper strips indicating that the higher R.H. aided in overcoming the influence of substrate dissimilarity.

These results indicated that exposure humidity should always be considered as an important factor in the design of a formaldehyde gas cycle for the sterilization of external surfaces. The preconditioning humidity may be regarded as a less influential factor, especially if elevated temperatures and a gas concentration greater than 1.0 mg/l is employed, since these could completely mask the influence of preconditioning moisture levels.

The results of experiments conducted on the inactivation of barrier-enclosed spores revealed that both moisture and temperature exerted significant influences on rate of kill. Data indicated that a relative humidity range of 30-50% would probably be permissible for the sterilization of barrier-protected microorganisms at a temperature of 25C. At 40C a relative humidity level of approximately 30% would be more effective than would atmospheres of 50 or 70% R.H.

Results of studies conducted to determine whether formaldehyde residues occurred following exposure indicated that little or no problem resulted with respect to the formation of residues in or on materials. Any residual formaldehyde that did result was easily dissipated with aeration at ambient conditions. Elevated temperatures of aeration would enhance the process of dissiptation of residuals. In field trials conducted at MSFC, ammonia gas was utilized to neutralize formaldehyde gas immediately upon the conclusion of exposure cycles. It was noted that more residual formaldehyde was detectable on materials exposed to ammonia than on materials exposed to formaldehyde only and removed before ammonia was used. This was apparently the result of precipitation of the neutral reaction product, hexamethylenetetramine, on the surfaces of the test materials used.

The initial series of field trials conducted at MSFC demonstrated the feasibility of large scale sterilization of spacecraft surfaces with dry formaldehyde gas at low concentrations. During the course of Trials 4-8, attention was directed towards an attempt to resolve the decreased inactivation of spores which occurred in these cycles. It became apparent that the spores used for the preparation of the spore strips employed in Trials 4-7 were approximately twice as resistive as those used in Trials 2, 3, and 8. This anomaly in combination with the empirically proven observation that pure ammonia gas used for neutralization was able to reverse the effect of formaldehyde provided a rationale for the lack of success achieved in the indicated trials. Although ammonia gas was used in Trial 3, it was thermally generated from ammonium carbonate powder and used at a lesser concentration than when pure ammonia gas was injected into the chamber from a cylinder.

Results suggested that when low concentrations of formaldehyde are used, ammonia may reverse biocidal effects if introduced into the sterilizing chamber too quickly. The exact relationship between formaldehyde exposure of materials and the introduction of neutralizing chemicals such as ammonia should be well established to avoid reversal of the biocidal activity of formaldehyde. The reason for good inactivation of spores packaged within various barriers may have been due to the good penetration of formaldehyde gas during the extended exposure cycle and limited or no penetrability of ammonia during the relatively short neutralizing period. Additional work on the kinetics of neutralization of formaldehyde gas by ammonia is indicated.

CONCLUSIONS

1. The feasibility of using formaldehyde-liberating synthetic resins or polymers for the sterilization of potting compounds, mated and occluded areas, and spacecraft surfaces has been demonstrated.

2. The detailed study of interrelated parameters of formaldehyde gas sterilization revealed that efficient cycle conditions can be developed for the sterilization of spacecraft components. It was determined that certain parameters were more important than others in the development of cycles for specific applications.

3. The use of formaldehyde gas for the sterilization of spacecraft components provides NASA with a highly efficient method which is inexpensive, reproducible, easily quantitated, materials compatible, operationally simple, generally non-hazardous and not thermally destructive.

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TABLES

TABLE I

COMPARATIVE BIOCIDAL ACTIVITY USING RTV-3140

Organism	Paraformaldehyde 1%	5%	10%	Melamine Formaldehyde 1.9%	9.5%	19%	Urea Formaldehyde 2.1%	10.6%	21%
Escherichia coli	10*	16	19	10	13	15	10	18	22
Staphylococcus aureus	24	43	46	15	28	36	21	31	38
Bacillus globigii	25	38	42	16	29	36	19	34	42
Serratia marcescens	13	20	25	10	14	19	13	20	24
Klebsiella pneumoniae	13	22	25	13	17	21	14	21	26

*Size of inhibition zone in millimeters.

TABLE II

COMPARATIVE BIOCIDAL ACTIVITY OF PARAFORMALDEHYDE, MELAMINE FORMALDEHYDE, AND UREA FORMALDEHYDE CONTAINED IN VARIOUS ELECTRONIC MATERIALS

Biocidal Materials	RTV 3140		Lucite		Chem Seal		Napcofoam		Eccofoam	
	E. coli	S. aureus	E. coli	S. aureus	E. coli	S. aureus	E. coli	S. aureus	E. coli	S. aureus
1% PF	10*	24	-	-	10	14	-	-	-	-
5% PF	16	43	21	38	11	22	10	10	17	32
9.5% MF	13	28	19	35	12	23	-	-	-	-
10.6% UF	18	31	-	-	13	19	-	-	10	12
Control	10	10	17	17	10	10	10	10	10	10

*Size of inhibition zone in millimeters.

TABLE III

Inactivation of Bacillus stearothermophilus Spores Inoculated onto Threads of Stainless Steel Spacecraft Components

Time (hours)	1.0% Paraformaldehyde*		0.1% Paraformaldehyde*	
	Test Pieces	Controls	Test Pieces	Controls
6	2.8×10^4	1.2×10^5		
12	6.5×10^1	1.6×10^5	4.8×10^4	1.4×10^5
24	2.9×10^1	1.0×10^5	2.3×10^3	1.2×10^5
48	6.7×10^0	1.6×10^5	4.4×10^0	1.5×10^5
72	--	--	0	1.6×10^5

*Suspended in trichloroethylene

TABLE IV

Inactivation of Bacillus stearothermophilus Spores Inoculated onto Threads of Aluminum Spacecraft Components

Time (hours)	1.0% Paraformaldehyde*		0.1% Paraformaldehyde*	
	Test Pieces	Controls	Test Pieces	Controls
6	4.1×10^4	1.1×10^5		
18	1.4×10^2	1.4×10^5		
24	0	9.2×10^4	1.2×10^1	1.4×10^5

*Suspended in trichloroethylene

TABLE V

Inactivation of Bacillus stearothermophilus Spores on Mated Surfaces of Spacecraft Tubing Connectors (stainless steel) at 60C with 0.1% or 1.0% Paraformaldehyde-Trichloroethylene Spray

Exposure Time, hours	0.1% Paraformaldehyde		1.0% Paraformaldehyde	
	Experimental	Control	Experimental	Control
6	5.74×10^4	1.8×10^5	--	--
18	1.40×10^4	1.8×10^5	--	--
24	1.62×10^4	1.8×10^5	6.9×10^3	1.65×10^5
48	6.55×10^3	1.4×10^5	4.4×10^4	1.38×10^5
72	2.85×10^3	2.0×10^5	7.4×10^3	1.70×10^5
144	1.95×10^3	1.0×10^5	--	--
192	--	--	6.7×10^3	5.5×10^4

TABLE VI

Effect of Preconditioning Humidity on the Formaldehyde[1] Sterilization of Spores[2] on Paper Strips at 40C

Exposure Relative Humidity (%)	Preconditioning Relative Humidity (%)	$D_{40C-1\ mg/l}$ value (minutes)
30	15	112
	30	106
	50	105
	70	102
50	15	72
	30	67
	50	62
	70	63
70	15	53
	30	54
	50	47
	70	42

[1]Formaldehyde concentration, 1.0 mg/l

[2]*Bacillus subtilis* var. *niger*, 1 x 10^6 spores per strip

TABLE VII

Effect of Preconditioning Humidity on the Formaldehyde[1] Sterilization of Spores[2] on Stainless Steel Strips at 40C

Exposure Relative Humidity (%)	Preconditioning Relative Humidity (%)	$D_{40C-1\ mg/l}$ value (minutes)
30	15	131
	30	196
	50	168
	70	154
50	15	91
	30	82
	50	100
	70	96
70	15	40
	30	40
	50	42
	70	44

[1]Formaldehyde concentration, 1.0 mg/l

[2]Bacillus subtilis var. niger, 1 x 10^6 spores per strip

TABLE VIII

Effect of Preconditioning Humidity on the Formaldehyde[1] Sterilization of Spores[2] on Paper Strips at 25C

Exposure Relative Humidity (%)	Preconditioning Relative Humidity (%)	$D_{25C-1\ mg/l}$ value (minutes)
30	15	390
	30	524
	50	168
	70	--
50	15	120
	30	126
	50	96
	70	54
70	15	42
	30	60
	50	99
	70	95

[1]Formaldehyde concentration, 1.0 mg/l

[2]Bacillus subtilis var. niger, 1 x 10^6 spores per strip

-- = Not tested

TABLE IX

Effect of Preconditioning Humidity on the Formaldehyde[1] Sterilization of Spores[2] on Stainless Steel Strips at 25C

Exposure Relative Humidity (%)	Preconditioning Relative Humidity (%)	$D_{25C-1\ mg/l}$ value (minutes)
30	15	1194
	30	1420
	50	690
	70	642
50	15	212
	30	264
	50	203
	70	242
70	15	60
	30	60
	50	137
	70	131

[1]Formaldehyde concentration, 1.0 mg/l

[2]*Bacillus subtilis* var. *niger*, 1 x 10^6 spores per strip

TABLE X

Influence of Humidity on Penetration of Formaldehyde Gas[1]
Through Barrier Materials at 25C and 40C

Barrier	25C			40C		
	$D_{25C-1\ mg/l}$ value (minutes)			$D_{40C-1\ mg/l}$ value (minutes)		
	30% R.H.	50% R.H.	70% R.H.	30% R.H.	50% R.H.	70% R.H.
3 mil polyethylene	*	120	312	88	244	286
6 mil polyethylene	*	72	420	93	219	1350
Cellophane	*	180	690	144	873	534
Cotton plug	*	*	40	91	68	84

*Zero survival at time of initial sample count

[1]Formaldehyde concentration, 1.0 mg/l

Challenge: Bacillus subtilis var. niger, 1×10^6 spores per strip

TABLE XI

Water Vapor in Air at Different Environmental Conditions

Relative Humidity (%)	Water Vapor (mg/l) at Indicated Temperature	
	25C	40C
30	6.92	15.36
50	11.53	25.61
70	14.14	35.85

TABLE XII

Summary of Data Obtained from Assays of Formaldehyde Residuals on Various Test Pieces

CYCLE PARAMETERS				Materials Tested	Amount of Residual Immediately After Exposure (µg/gram)[1]	Amount of Residual After 1 Week Holding
% R.H.	Temp. (C)	Exposure Time, hrs.	Gas Conc. (mg/l)			
70	25	18	1.1	Cotton swab	338	0
				Glass strip	0	-
				Steel strip	0	-
				Plastic strip	0	-
70	25	3	1.1	Cotton swab	1960	0
				Glass strip	0	-
				Steel strip	0	-
				Plastic strip	0	-
70	40	3	1.1	Cotton swab	1040	0
				Glass strip	0	-
				Steel strip	0	-
				Plastic strip	0	-
70	40	1.5	1.1	Cotton swab	1140	0
				Glass strip	0	-
				Steel strip	0	-
				Plastic strip	0	-
50	40	1.5	1.1	Cotton swab	1136	0
				Glass strip	0	-
				Steel strip	0	-
				Plastic strip	0	-
50	25	3.0	1.1	Cotton swab	840	0
				Glass strip	0	-
				Steel strip	0	-
				Plastic strip	0	-
50	47	18	10.0	Latex rubber	55	0
				Silicone rubber	0	-
50	25	18	1.0	Filter paper	8000	0
70	25	6	1.0	Filter paper	4900	0
30	25	3	1.0	Filter paper	570	0
50	43	18	8.4	Polystyrene[2]	24	<1
50	50	18	6.4	Polystyrene[2]	30	<1
50	50	18	18.0	Polystyrene[2]	29	5
50	50	18	18.0	Polystyrene[2]	55	<1

[1]Measurements of materials tested:
Stainless steel: 15 x 50 mm, 3.96g
Glass: 15 x 50 mm, 2.75g
Polypropylene: 15 x 50 mm, 1.21g
Cotton swabs: 0.3g
Filter paper: 0.3g
Latex rubber: 1.0g
Silicone rubber: 1.0g
Polystyrene: 1.0g

[2]Enclosed in paper barrier

TABLE XIII

Summary of Results from All Trials on Sterilization of the TFS with Formaldehyde Gas

Trial	Percent Inactivation of Spores				CONDITIONS OF EXPOSURE*			
	10^4	10^5	10^6	10^7	Gas Conc. (mg/l)	Temp. (%)	Relative Humidity (%)	Time (hours)
1	-	-	-	-	1.08	58	55	6
2	100	100	100	92	1.27	66	45	12
3**	100	100	100	100	1.39	86	45	6
4**	100	88	94	60	1.41	88	47	6
5**	92	70	60	38	1.62	88	63	6
6**	70	48	30	14	1.47	92	47	6
7**	96	68	41	6	1.60	86	48	12
8a	98	100	90	82	2.10	90	47	12
8b**	90	100	75	20	2.10	90	47	12

*Average values for gas concentration, temperature, and relative humidity

**Test samples exposed to formaldehyde and neutralizer (ammonia): all others exposed to formaldehyde only

TABLE XIV

Trial 2--Penetrability of Formaldehyde Gas Through
Various Barrier Materials
(MSFC Trial)

Type of Barrier Material	Number of Spores* per Strip			
	10^4	10^5	10^6	10^7
Polyethylene film, 1 mil	0/1**	0/1	0/1	0/1
Polyethylene film, 2 mil	0/1	0/1	0/1	0/1
Polyethylene film, 4 mil	0/1	0/1	0/1	0/1
Polyethylene film, 6 mil	0/1	0/1	0/1	0/1
Latex rubber, single thickness	-	-	5/5	-

*Bacillus subtilis var. niger

**Number sterile/number tested

- = Not tested

TEST CONDITIONS: Gas concentration, 1.27 mg/l; Temperature, 66F;
% Relative humidity, 45; Exposure time, 12 hours

TABLE XV

Trial 4--Penetrability of Formaldehyde Gas Through
Various Barrier Materials
(MSFC Trial)

Type of Barrier Material	Number of Spore per Strip			
	10^4	10^5	10^6	10^7
Polyethylene film, 2 mil	4/4**	4/4	3/4	3/6
Polyethylene film, 4 mil	4/4	4/4	4/4	2/6
Polyethylene film, 6 mil	1/5	1/5	4/6	0/6
Cellophane, double thickness	0/6	2/6	0/6	0/6
Latex rubber, single thickness	-	-	2/6	-
Latex rubber, double thickness	-	-	2/6	-
Cloth-covered urethane foam	2/2	2/2	1/2	0/2

*Bacillus subtilis var. niger

**Number sterile/number tested

- = Not tested

TEST CONDITIONS: Gas concentration, 1.41 mg/l; Temperature, 88F;
% Relative humidity, 47; Exposure time, 6 hours

TABLE XVI

Trial 5--Penetrability of Formaldehyde Gas Through
Various Barrier Materials
(MSFC Trial)

Type of Barrier Material	Number of Spores* per Strip			
	10^4	10^5	10^6	10^7
Polyethylene film, 2 mil	4/4**	4/4	1/4	1/4
Polyethylene film, 4 mil	4/4	3/4	2/4	2/4
Polyethylene film, 5 mil	0/4	1/4	0/4	1/4
Polyethylene film, 6 mil	1/4	0/4	0/4	0/4
Cellophane, double thickness	4/4	4/4	2/4	1/4
Latex rubber, single thickness	-	-	8/8	-
Latex rubber, double thickness	-	-	1/4	-
Cloth-covered urethane foam	2/2	1/2	0/2	1/2
Glassine paper	4/4	3/4	1/4	1/4
Cotton plug	4/4	4/4	0/4	1/4
Polystyrene flasks, screwcapped	4/4	4/4	3/4	2/4

*Bacillus subtilis var. niger

**Number sterile/number tested

- = Not tested

TEST CONDITIONS: Gas concentration, 1.62 mg/l; Temperature, 88F;
% Relative humidity, 63; Exposure time, 6 hours

TABLE XVII

Trial 6--Penetrability of Formaldehyde Gas Through Various Barrier Materials (MSFC Trial)

Type of Barrier Material	Number of Spores* per Strip			
	10^4	10^5	10^6	10^7
Polyethylene film, 2 mil	4/4**	4/4	4/4	3/4
Polyethylene film, 3 mil	4/4	4/4	4/4	1/4
Polyethylene film, 4 mil	4/4	4/4	4/4	4/4
Polyethylene film, 5 mil	4/4	4/4	4/4	0/4
Polyethylene film, 6 mil	1/4	2/4	1/4	0/4
Cellophane, double thickness	0/4	0/4	0/4	0/4
Latex rubber, single thickness	-	-	0/4	-
Latex rubber, double thickness	-	-	0/4	-
Glassine paper	4/4	4/4	3/4	0/4
Cotton plug	4/4	4/4	4/4	4/4
Polystyrene flasks, screwcapped	0/4	3/4	2/4	2/4

*Bacillus subtilis var. niger

** Number sterile/number tested

- = Not tested

TEST CONDITIONS: Gas concentration, 1.47 mg/l; Temperature, 92F; % Relative humidity, 47; Exposure time, 6 hours

TABLE XVIII

Trial 7--Penetrability of Formaldehyde Gas Through Various Barrier Materials (MSFC Trial)

Type of Barrier Material	Number of Spores* per Strip			
	10^4	10^5	10^6	10^7
Polyethylene film, 2 mil	4/4**	4/4	1/4	2/4
Polyethylene film, 4 mil	4/4	4/4	1/4	2/4
Polyethylene film, 5 mil	0/4	2/4	0/4	0/4
Polyethylene film, 6 mil	0/4	1/4	0/4	0/4
Cellophane, double thickness	0/4	0/4	0/4	0/4
Latex rubber, single thickness	-	-	2/4	-
Latex rubber, double thickness	-	-	1/4	-
Glassine paper	3/4	2/4	0/4	1/4
Cotton plug	4/4	2/4	2/4	1/4
Polystyrene flasks, screwcapped	4/4	4/4	2/4	2/4

*Bacillus subtilis var. niger

**Number sterile/number tested

- = Not tested

TEST CONDITIONS: Gas concentration 1.60 mg/l; temperature, 86F; % Relative humidity, 48; Exposure time, 12 hours

TABLE XIX

Trial 8--Penetrability of Formaldehyde Gas Through Various Barrier Materials (MSFC Trial)

Type of Barrier Material	Number of Spores* per Strip			
	10^4	10^5	10^6	10^7
Polyethylene film, 2 mil	4/4**	4/4	4/4	4/4
Polyethylene film, 4 mil	4/4	4/4	4/4	4/4
Polyethylene film, 5 mil	4/4	4/4	4/4	4/4
Polyethylene film, 6 mil	3/4	3/4	3/4	4/4
Cellophane, double thickness	0/4	0/4	0/4	1/4
Latex rubber, single thickness	-	-	4/4	-
Latex rubber, double thickness	-	-	8/8	-
Glassine paper	4/4	4/4	3/4	4/4
Cotton plug	3/4	4/4	4/4	4/4

*Bacillus subtilis var. niger

**Number sterile/number tested

- = Not tested

TEST CONDITIONS: Gas concentration 2.08 mg/l; Temperature, 90F, % Relative humidity, 47; Exposure time, 12 hours

FIGURES

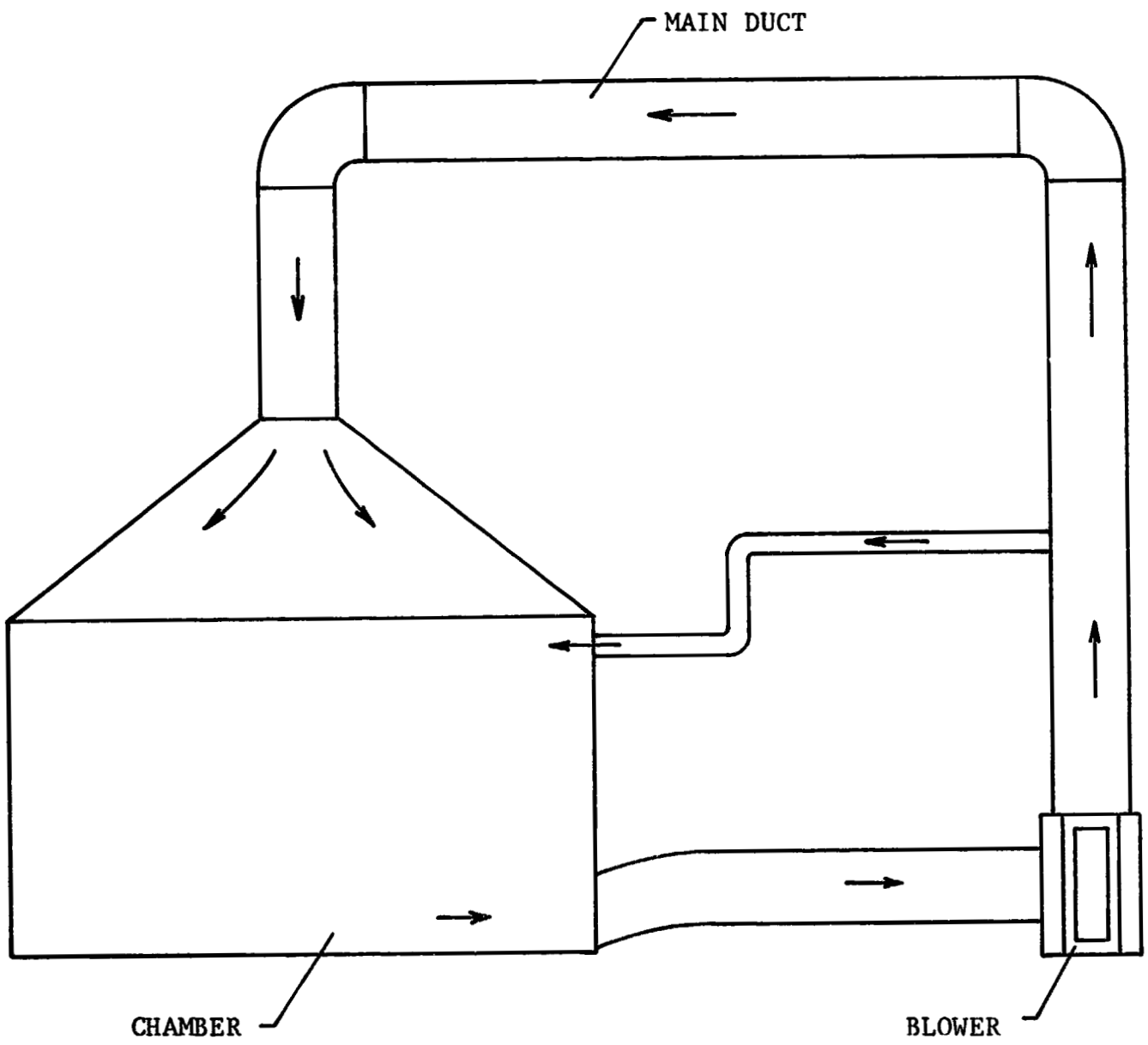


FIGURE 1: OVERALL VIEW OF CHAMBER AND DUCTS
PRIOR TO MODIFICATIONS

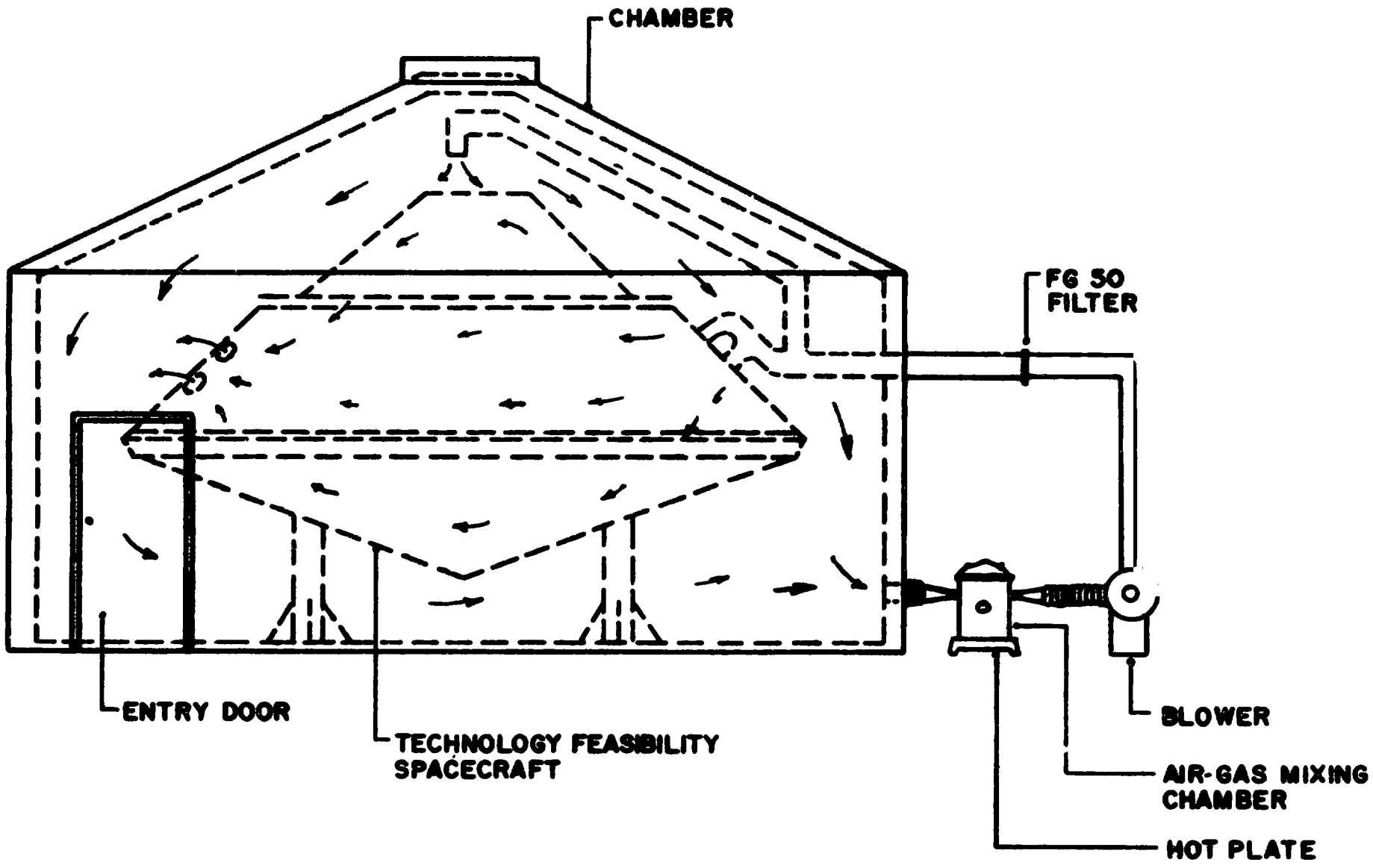


FIGURE 2: OVERALL VIEW OF GAS CHAMBER WITH MODIFIED DUCT SYSTEM AND ASSOCIATED EQUIPMENT.

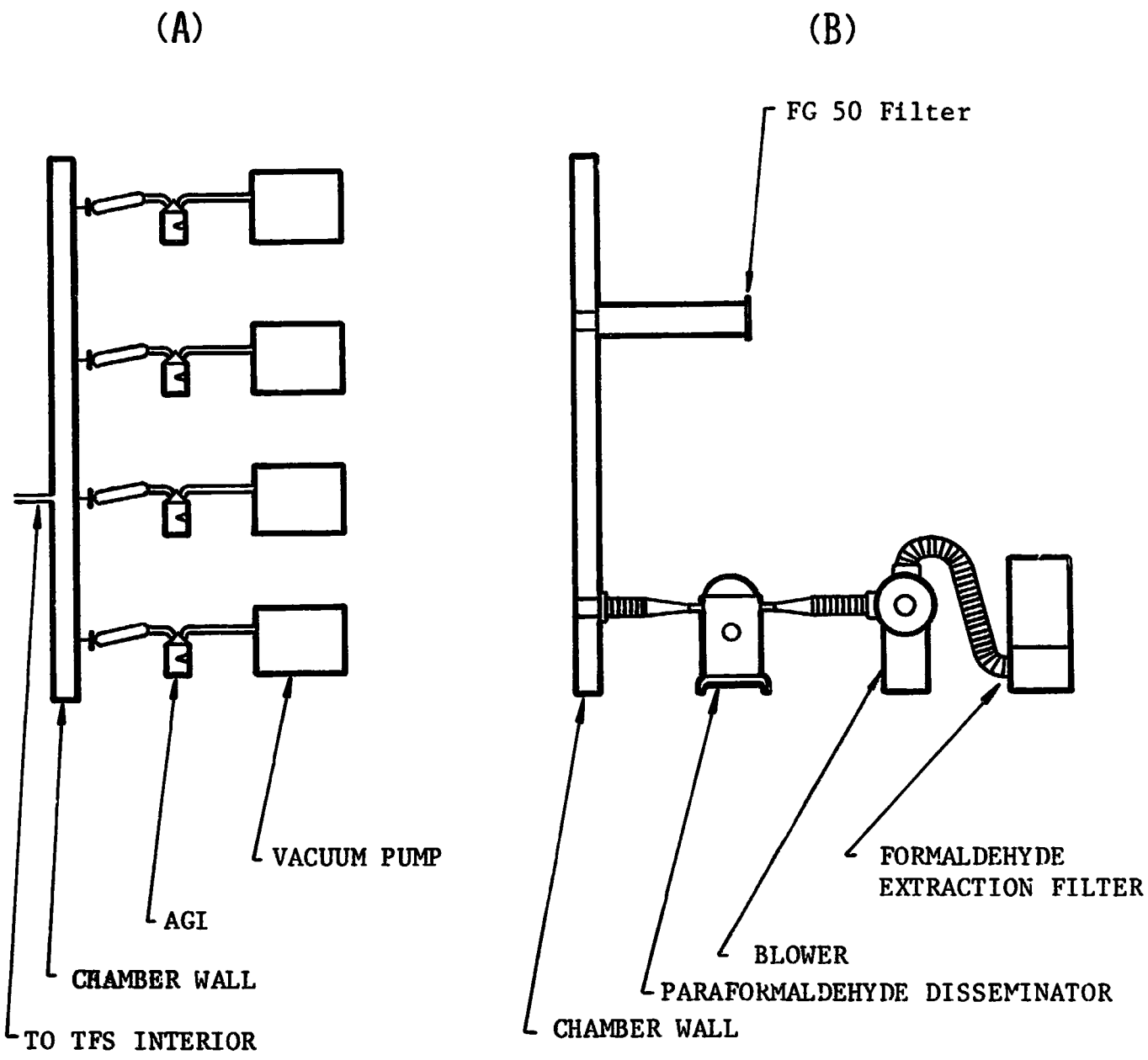


FIGURE 3: GAS SAMPLING (A) AND EXHAUST SYSTEMS (B)

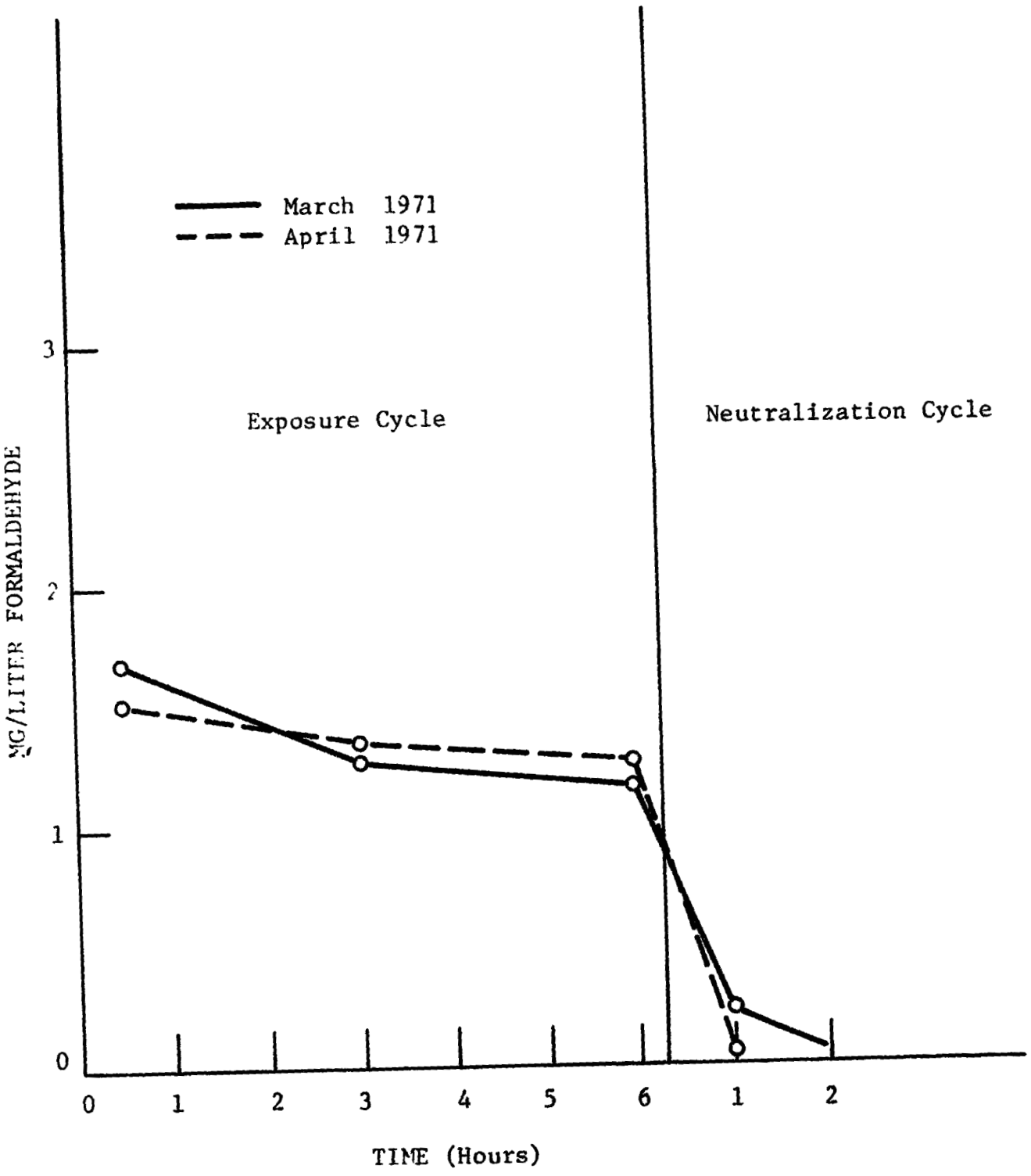


FIGURE 4: FORMALDEHYDE CONCENTRATIONS DURING EXPOSURE AND AMMONIA NEUTRALIZATION CYCLES

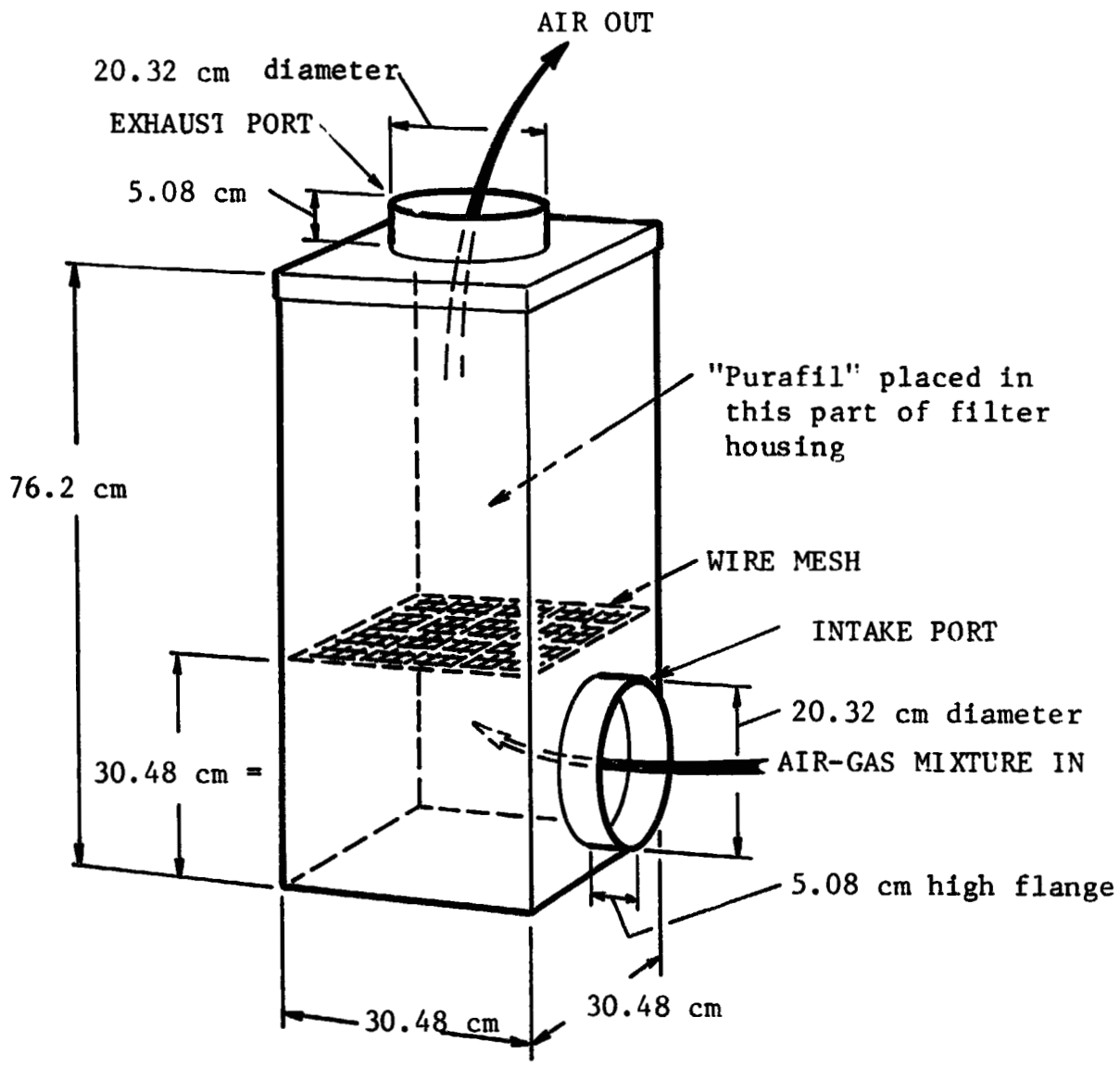


FIGURE 5: FORMALDEHYDE EXTRACTION FILTER

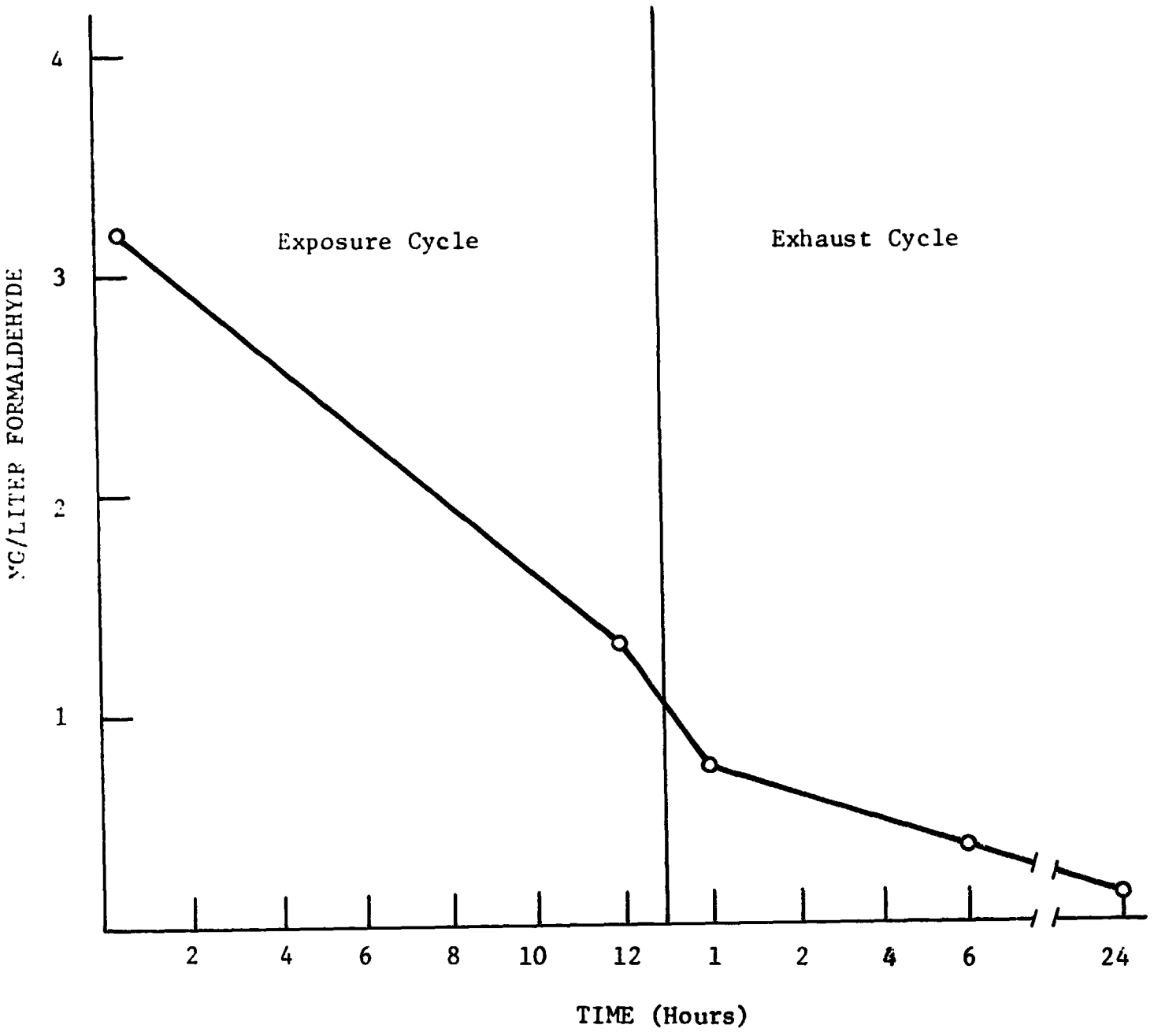


FIGURE 6: FORMALDEHYDE GAS CONCENTRATIONS
DURING EXPOSURE AND EXHAUST CYCLES

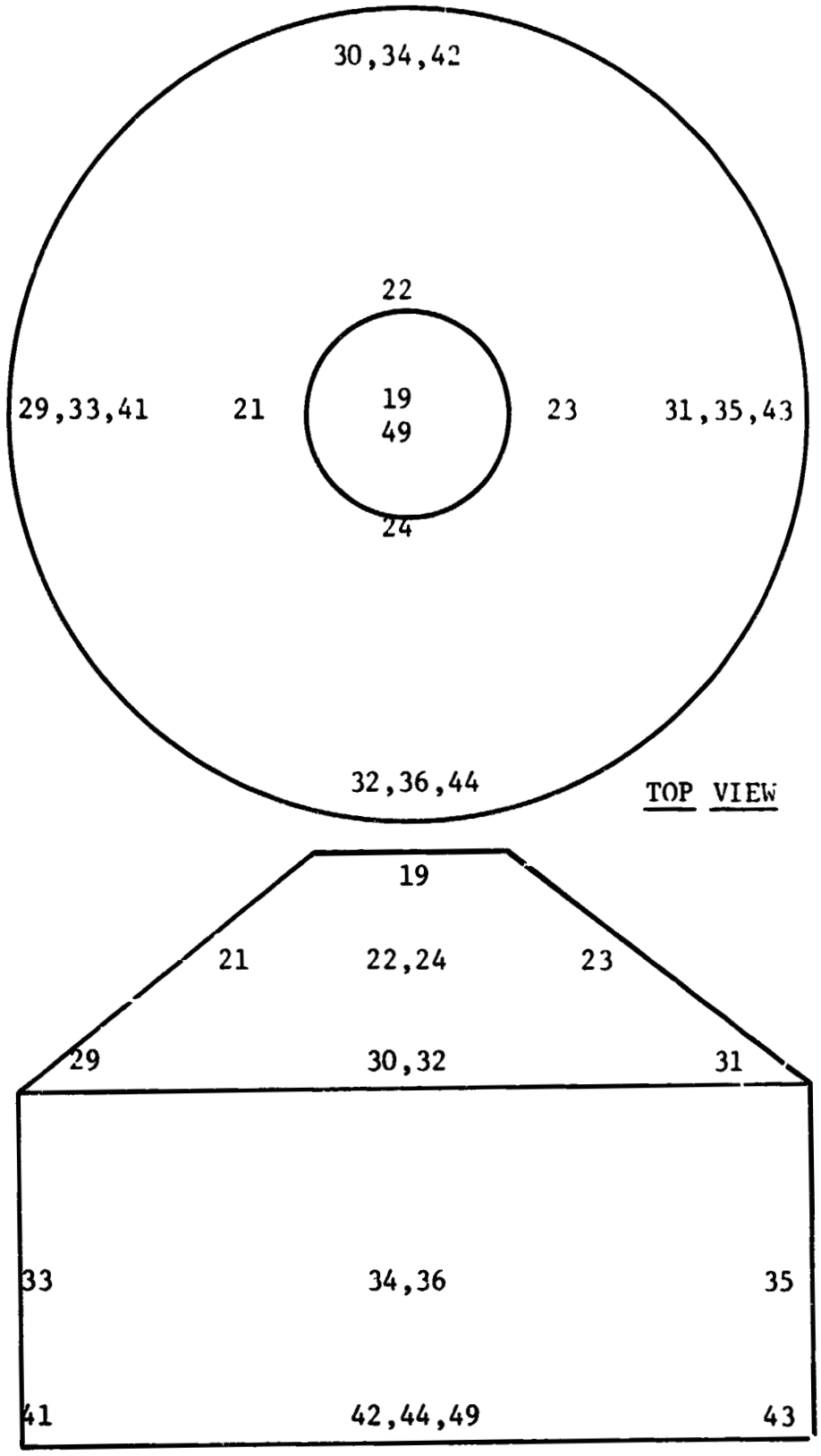


FIGURE 7: LOCATIONS OF SPORE STRIP SETS
ON CHAMBER WALL, CEILING AND FLOOR

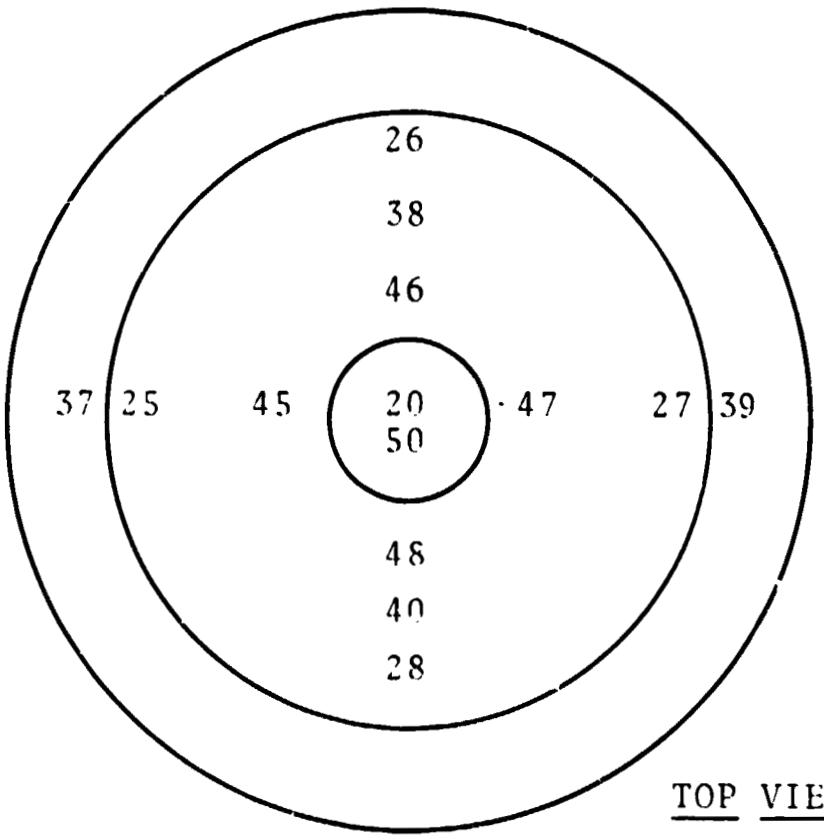
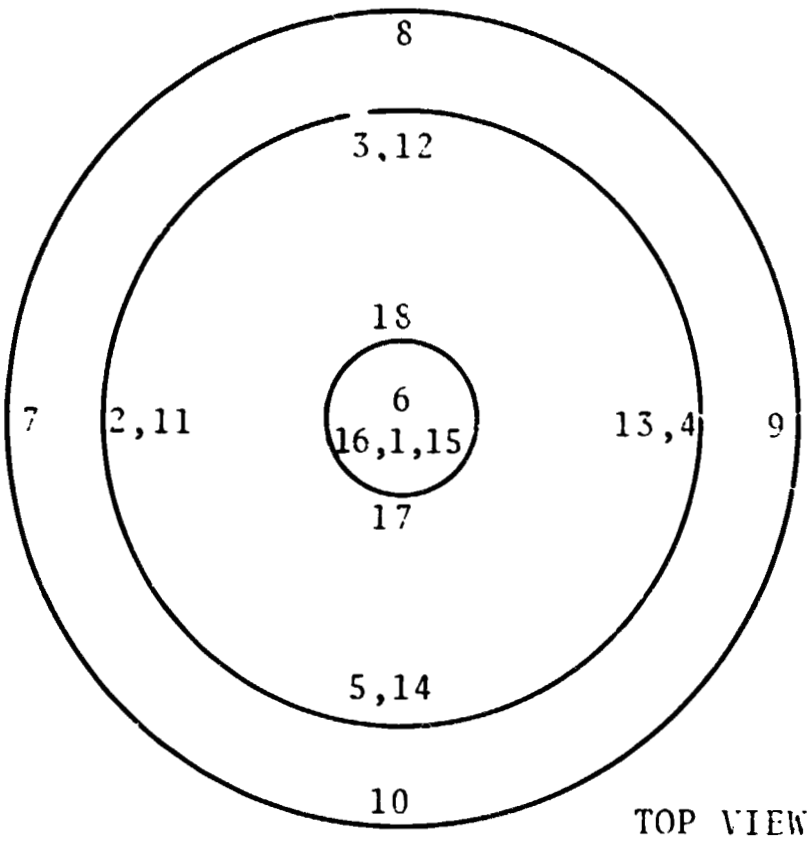


FIGURE 8: LOCATIONS OF SPORE STRIP SETS ON EXTERIOR OF TECHNOLOGY FEASIBILITY SPACECRAFT



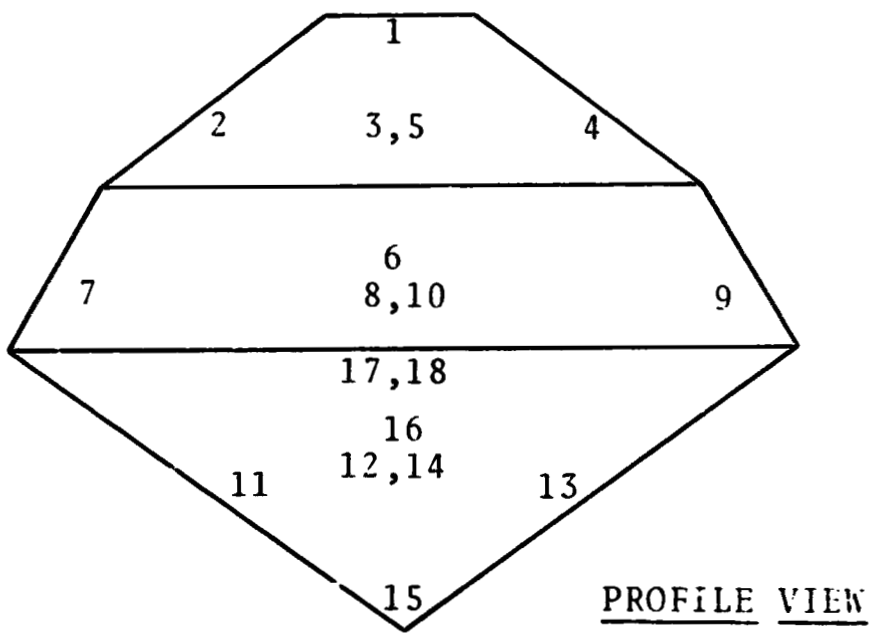


FIGURE 9: LOCATIONS OF SPORE STRIP SETS INSIDE TECHNOLOGY FEASIBILITY SPACECRAFT

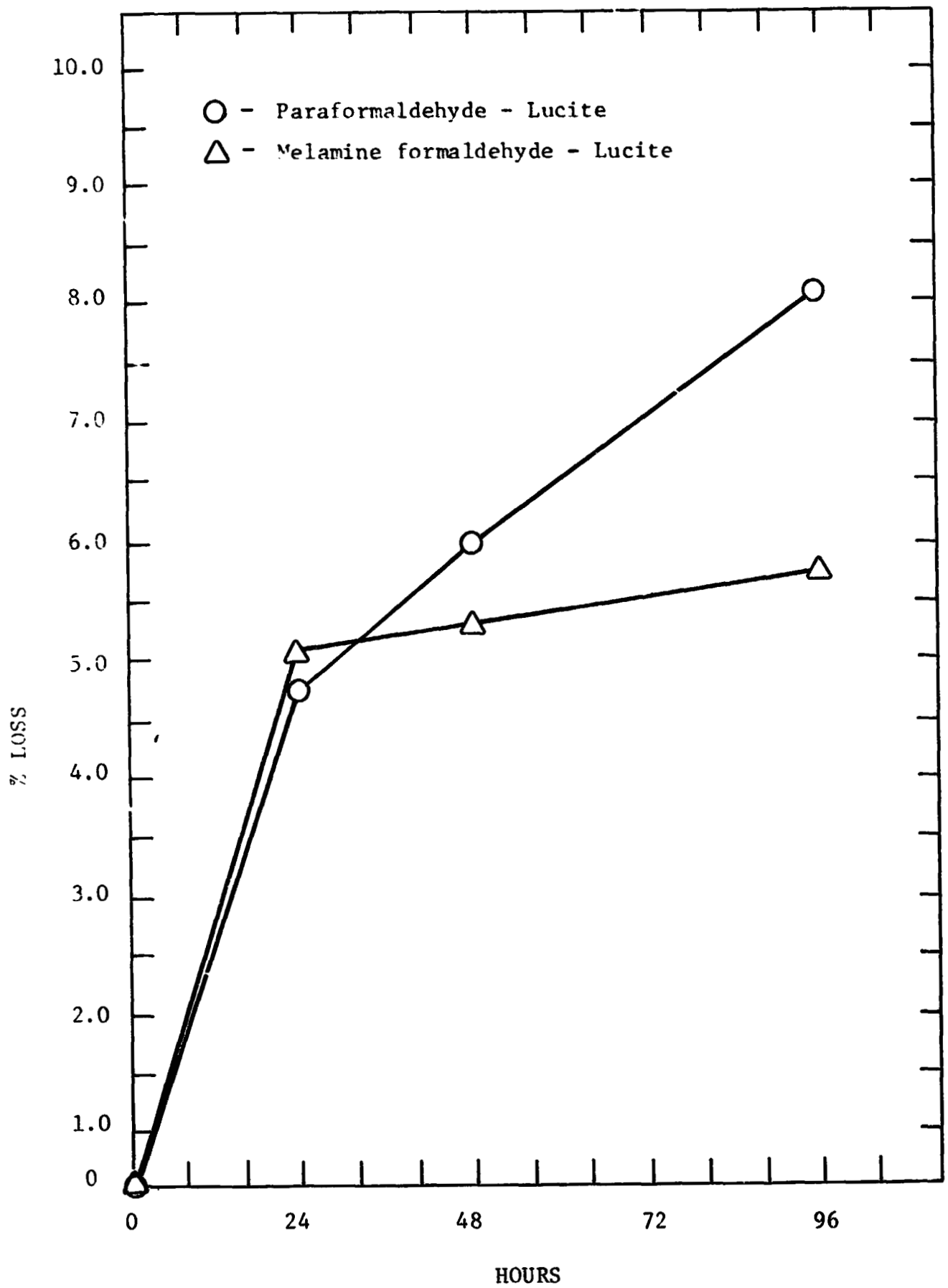


FIGURE 10: RELEASE OF FORMALDEHYDE FROM PARAFORMALDEHYDE (5%) AND MELAMINE FORMALDEHYDE (9.5%) CONTAINED IN LUCITE DISCS HEATED AT 60°C

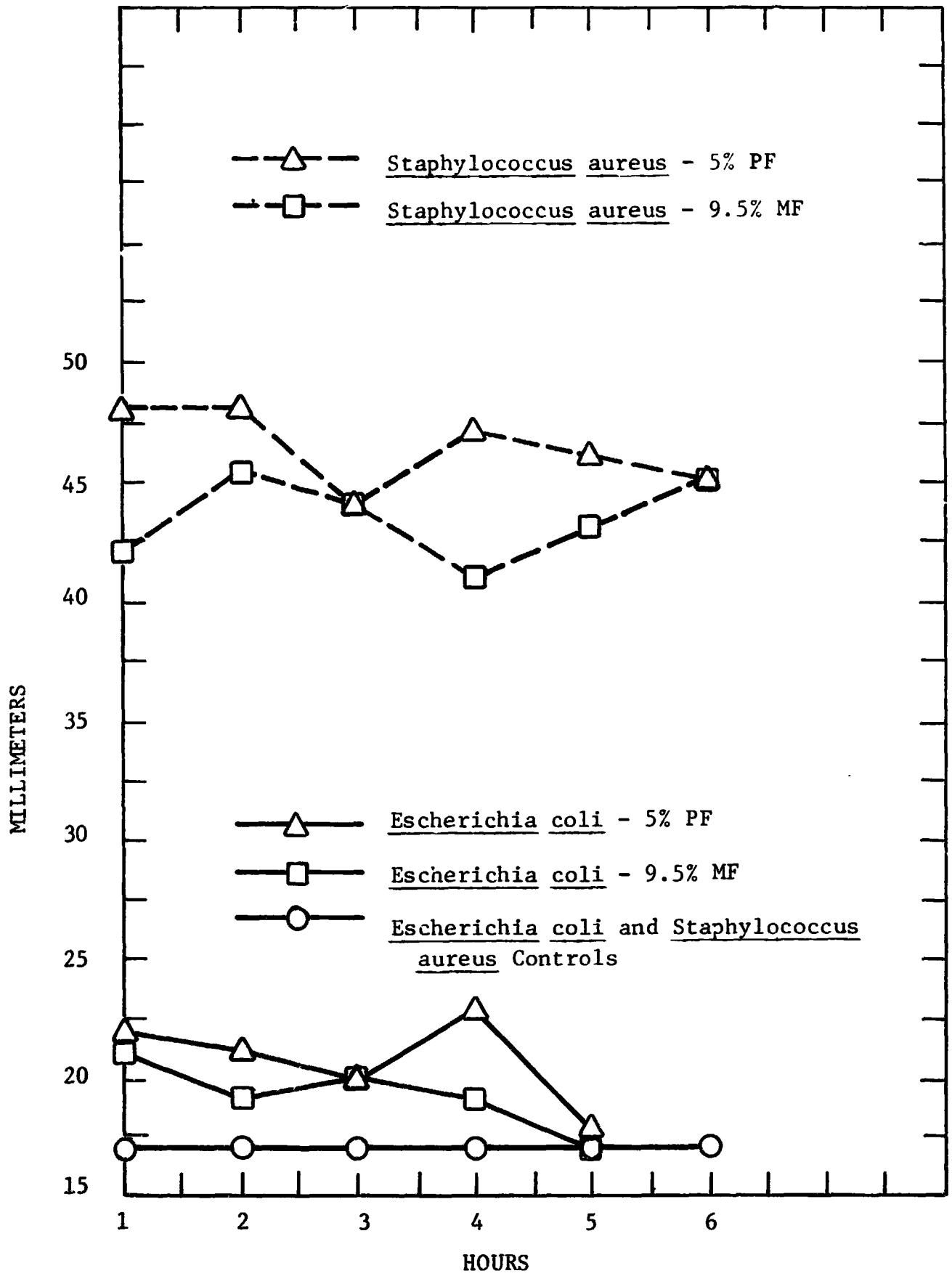


FIGURE 11: ZONES OF INHIBITION PRODUCED BY LUCITE DISCS CONTAINING 5% PARAFORMALDEHYDE (PF) OR 9.5% MELAMINE FORMALDEHYDE (MF) PREVIOUSLY HEATED FOR 1-6 HOURS AT 60C.

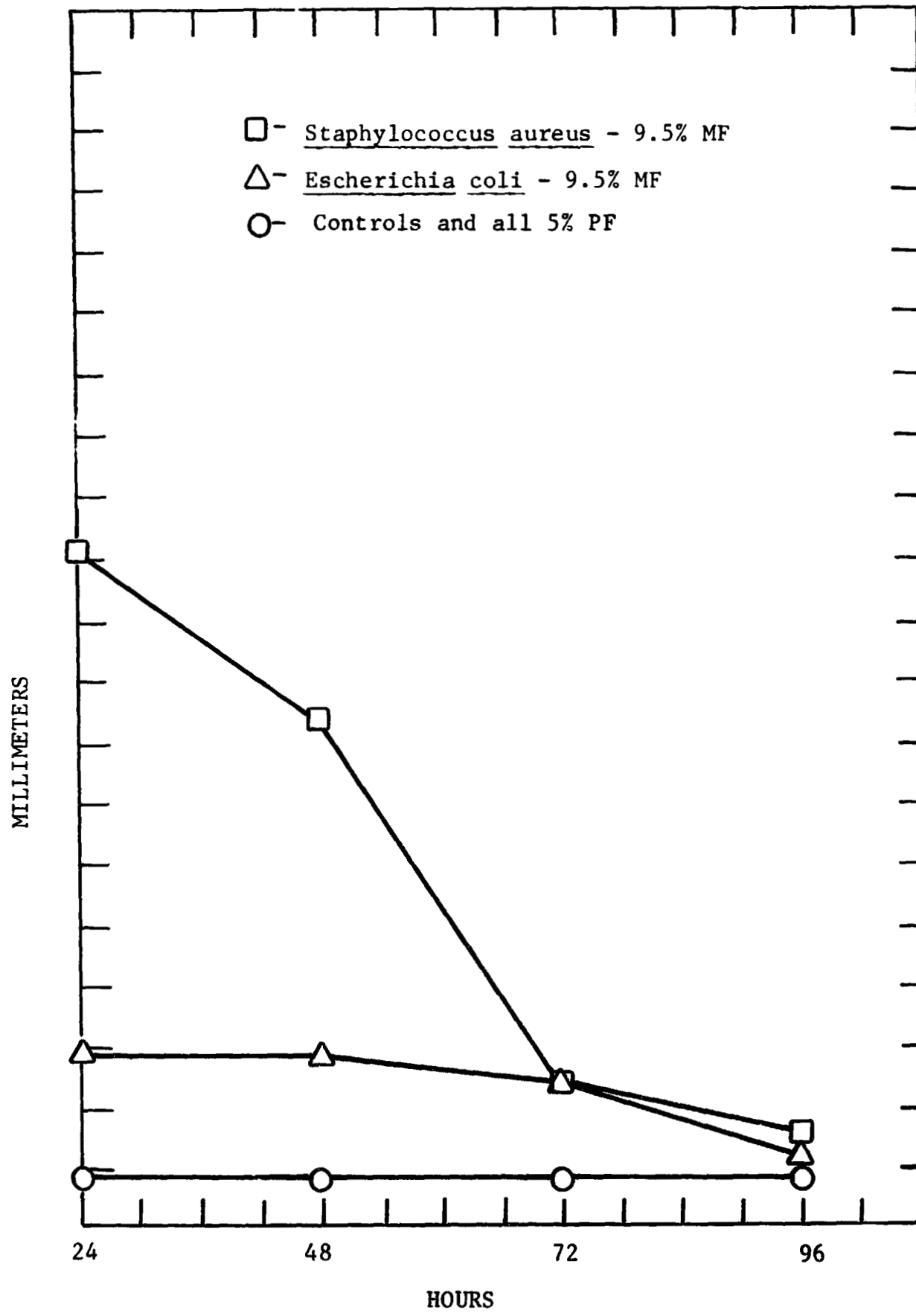


FIGURE 12: ZONES OF INHIBITION PRODUCED BY LUCITE DISCS CONTAINING 5% PARAFORMALDEHYDE OR 9.5% MELAMINE FORMALDEHYDE PREVIOUSLY HEATED FOR 24-96 HOURS AT 60C.

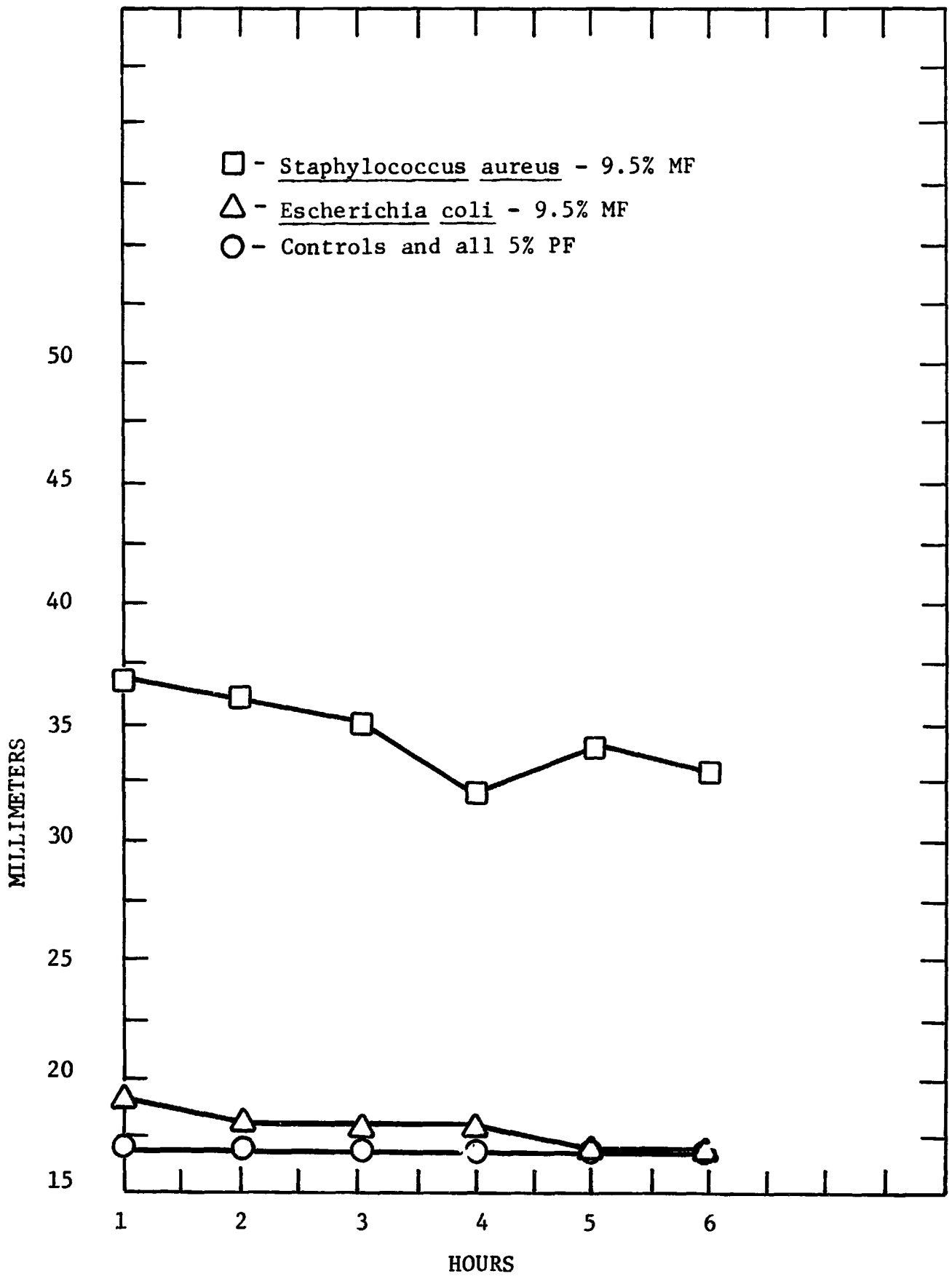


FIGURE 13:ZONES OF INHIBITION PRODUCED BY LUCITE DISCS CONTAINING 5% PARAFORMALDEHYDE OR 9.5% MELAMINE FORMALDEHYDE PREVIOUSLY HEATED FOR 1-6 HOURS AT 90C.

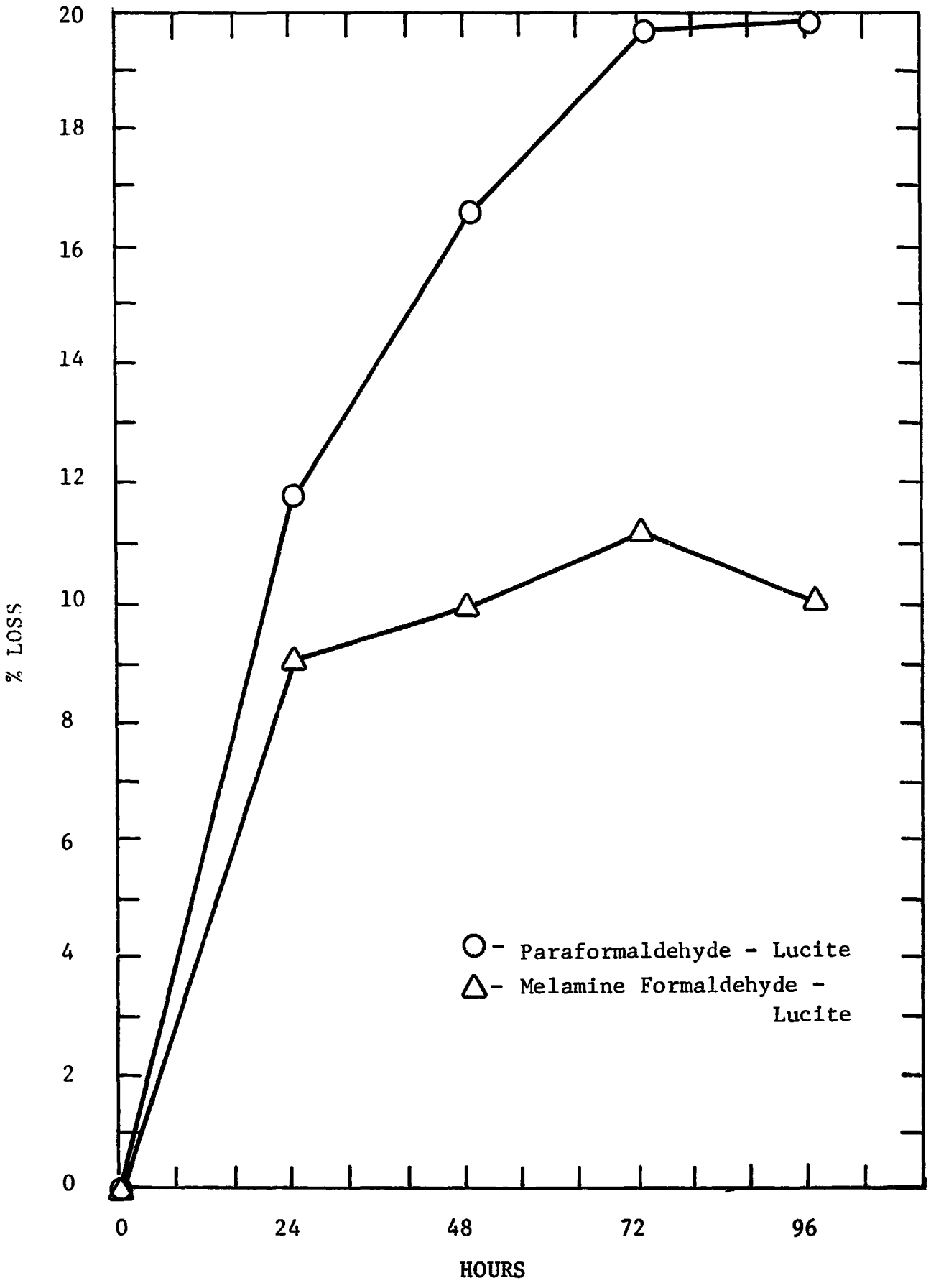


FIGURE 14: RELEASE OF FORMALDEHYDE FROM PARAFORMALDEHYDE (5%) AND MELAMINE FORMALDEHYDE (9.5%) CONTAINED IN LUCITE DISCS HEATED AT 90°C

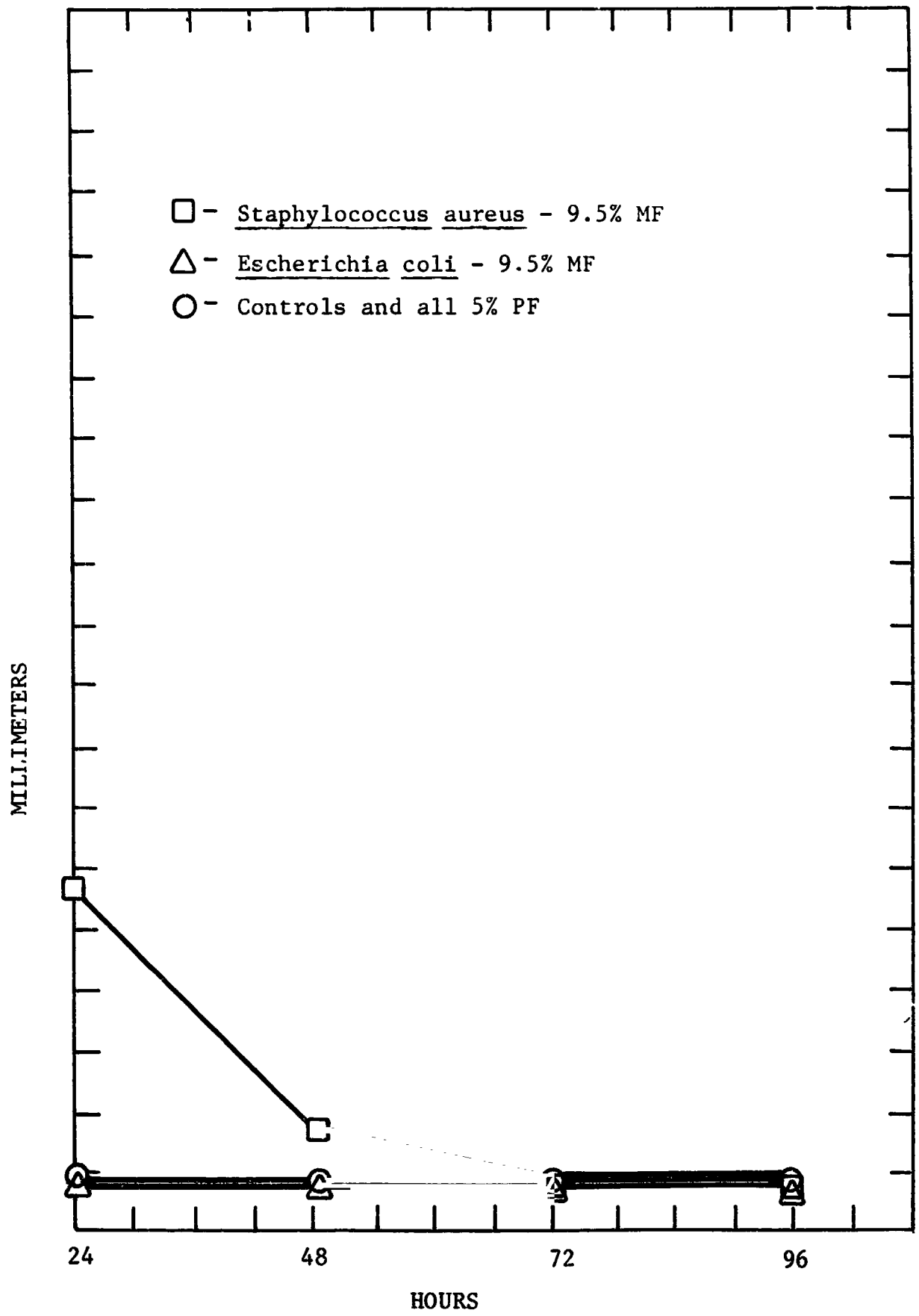


FIGURE 15: ZONES OF INHIBITION PRODUCED BY LUCITE DISCS CONTAINING 5% PARAFORMALDEHYDE OR 9.5% MELAMINE FORMALDEHYDE PREVIOUSLY HEATED FOR 24-96 HOURS AT 90C.

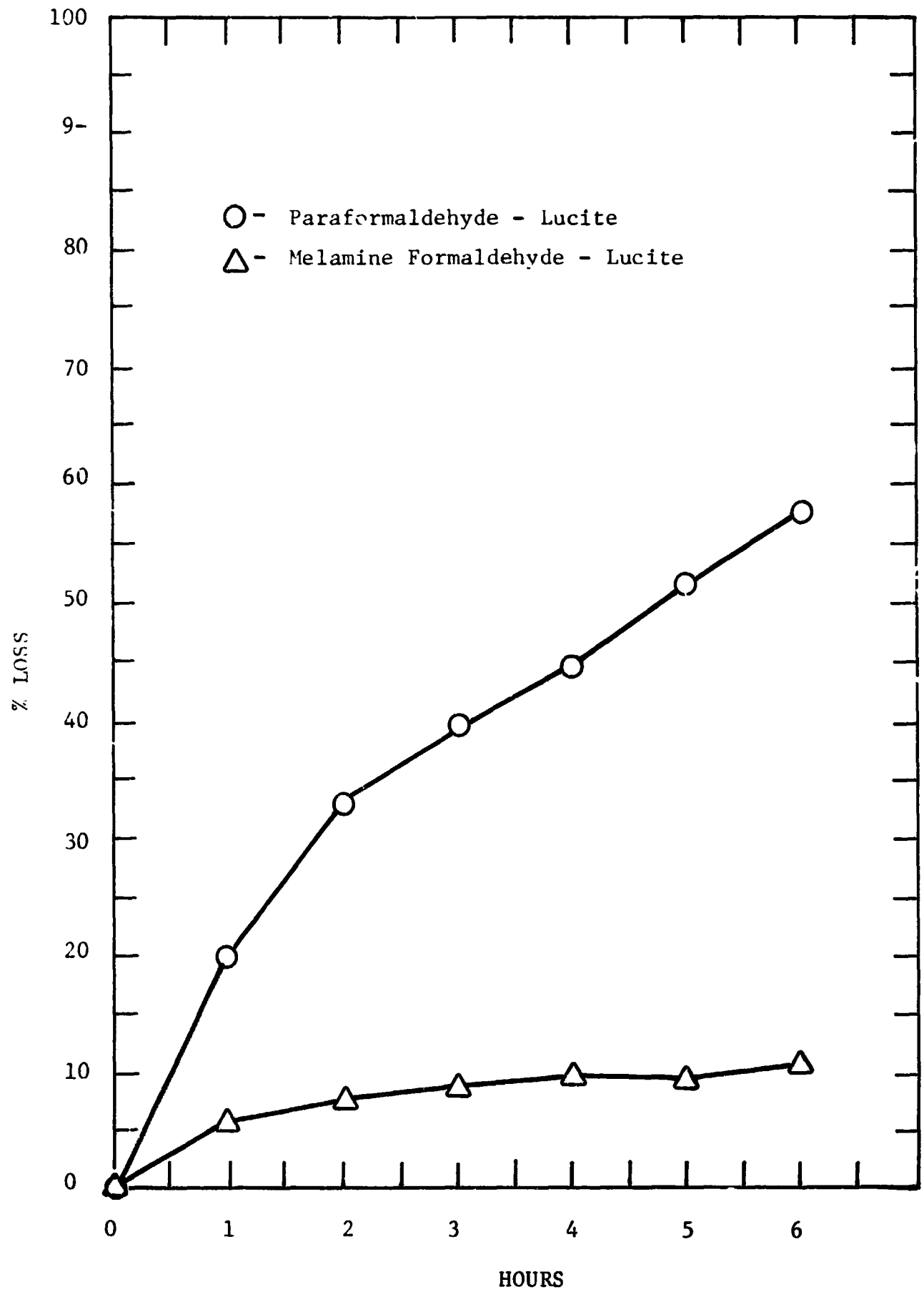


FIGURE 16: RELEASE OF FORMALDEHYDE FROM PARAFORMALDEHYDE (5%) AND MELAMINE FORMALDEHYDE (9.5%) CONTAINED IN LUCITE DISCS HEATED AT 125°C.

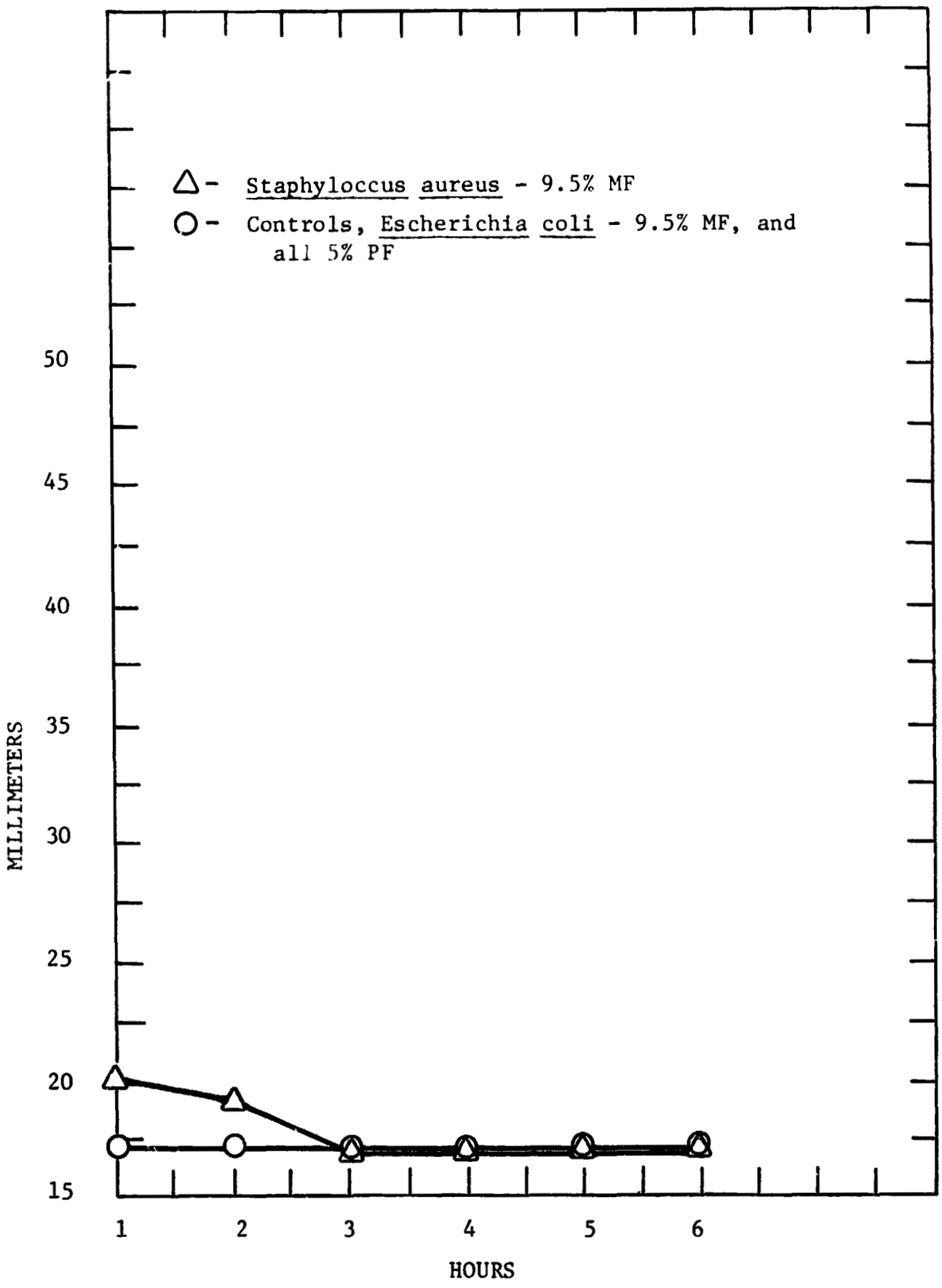


FIGURE 17: ZONES OF INHIBITION PRODUCED BY LUCITE DISCS CONTAINING 5% PARAFORMALDEHYDE OR 9.5% MELAMINE FORMALDEHYDE PREVIOUSLY HEATED FOR 1-6 HOURS AT 125C.

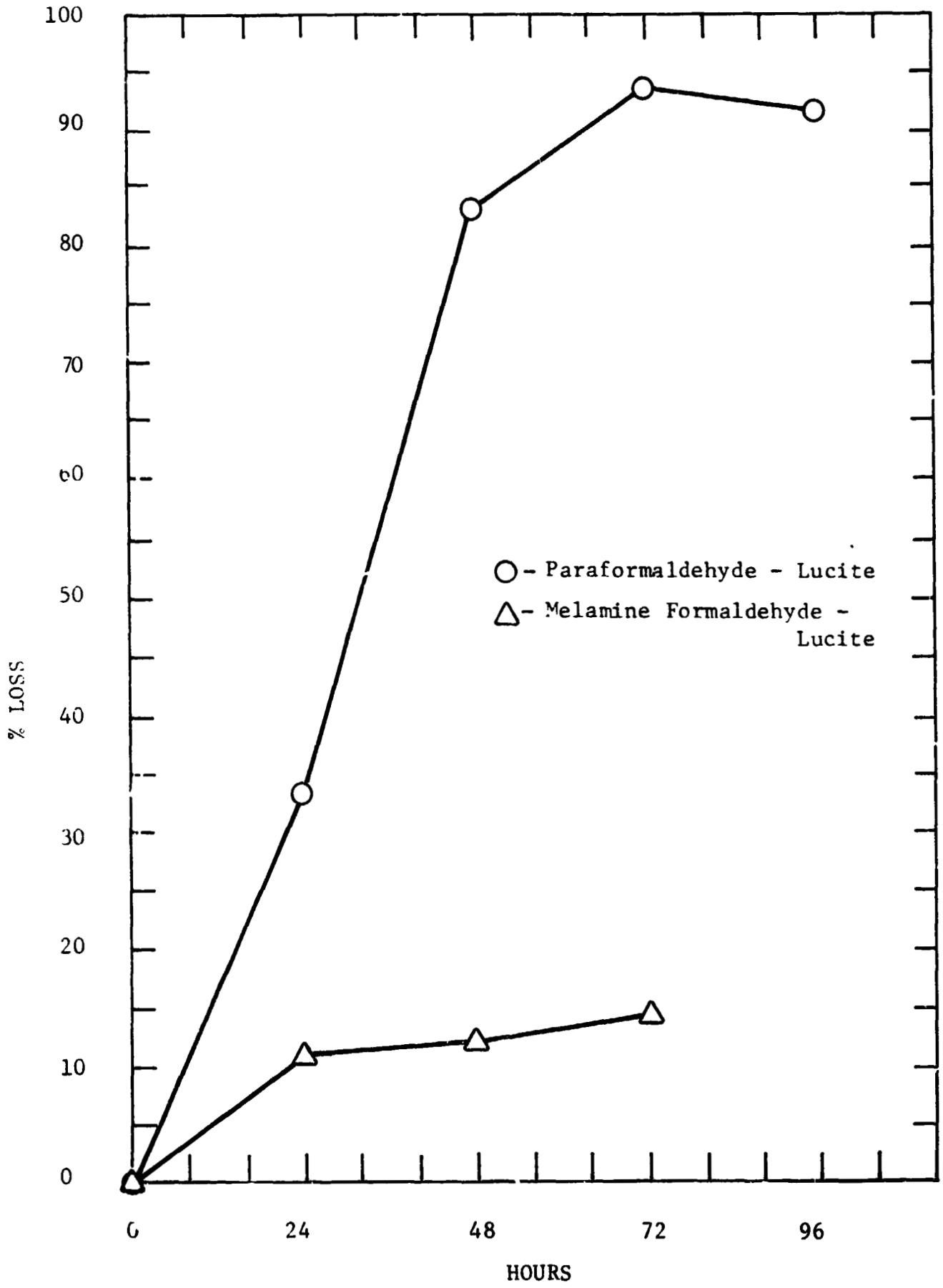


FIGURE 18: RELEASE OF FORMALDEHYDE FROM PARAFORMALDEHYDE (5%) AND MELAMINE FORMALDEHYDE (9.5%) CONTAINED IN LUCITE DISCS HEATED AT 125°C.

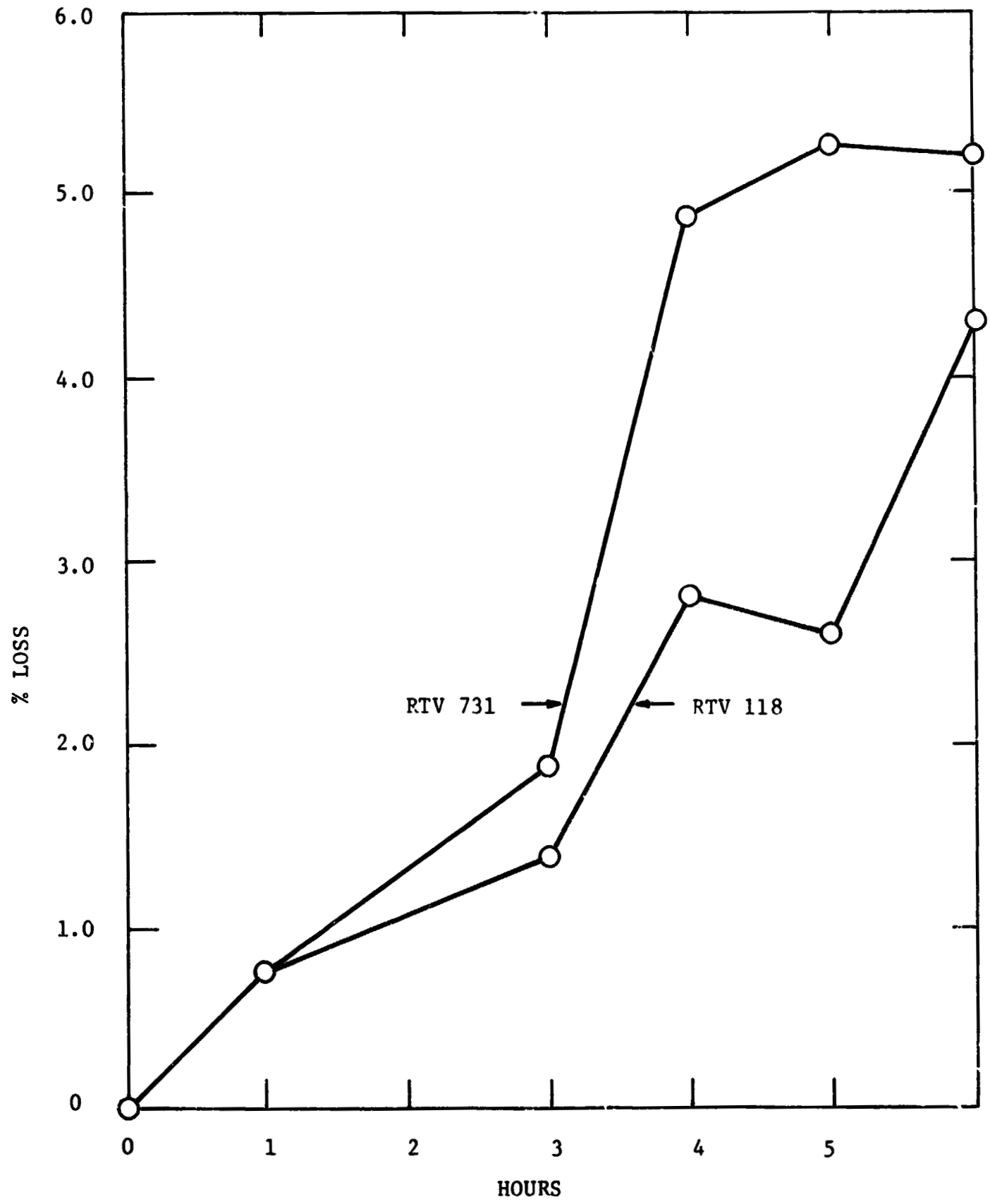


FIGURE 19: RELEASE OF FORMALDEHYDE FROM PARAFORMALDEHYDE (5%) CONTAINED IN DISCS OF RTV DURING HEATING AT 60°C.

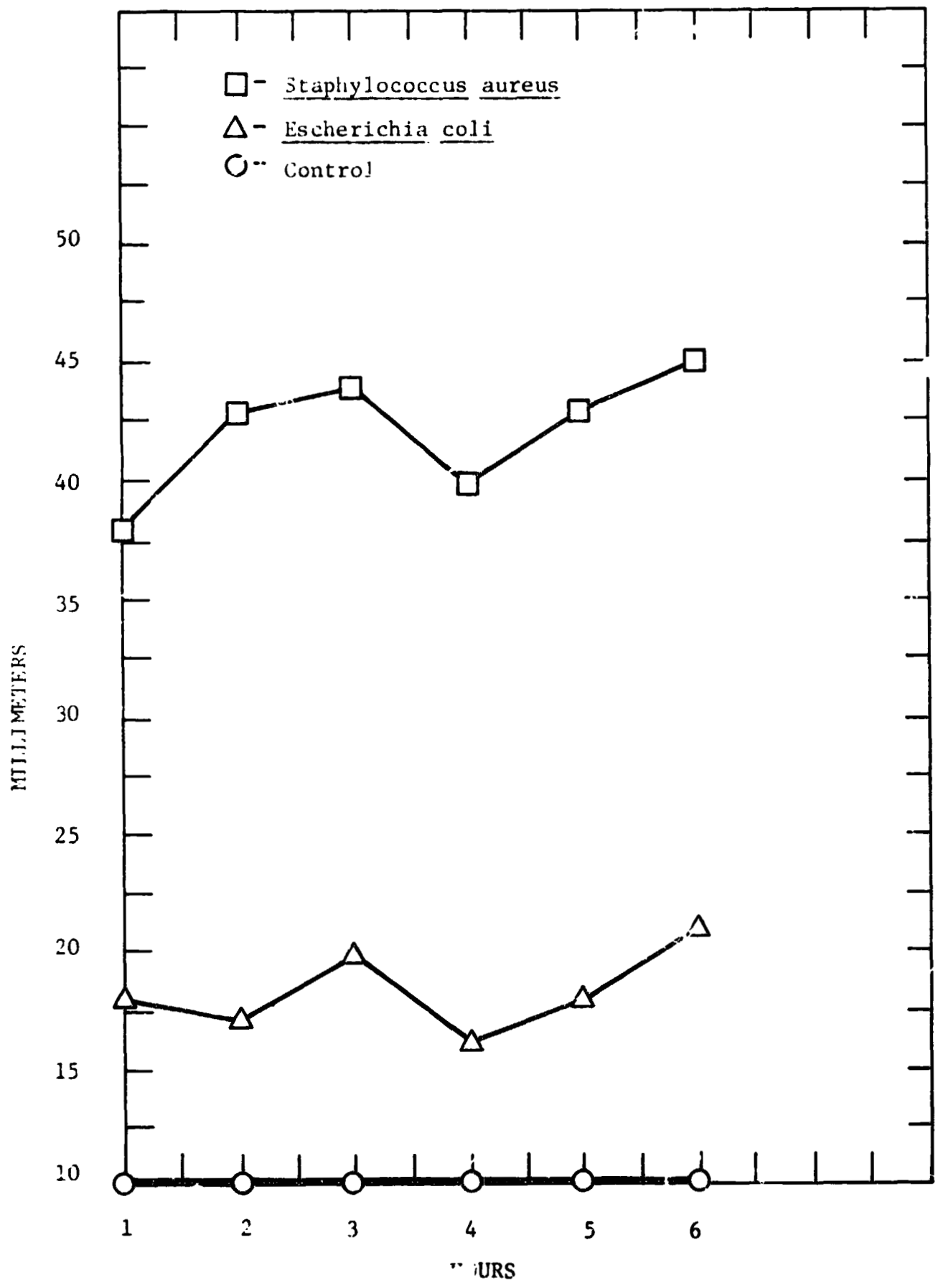


FIGURE 20: ZONES OF INHIBITION PRODUCED BY RTV 118 DISCS CONTAINING 5% PARAFORMALDEHYDE PREVIOUSLY HEATED FOR 1-6 HOURS AT 60C.

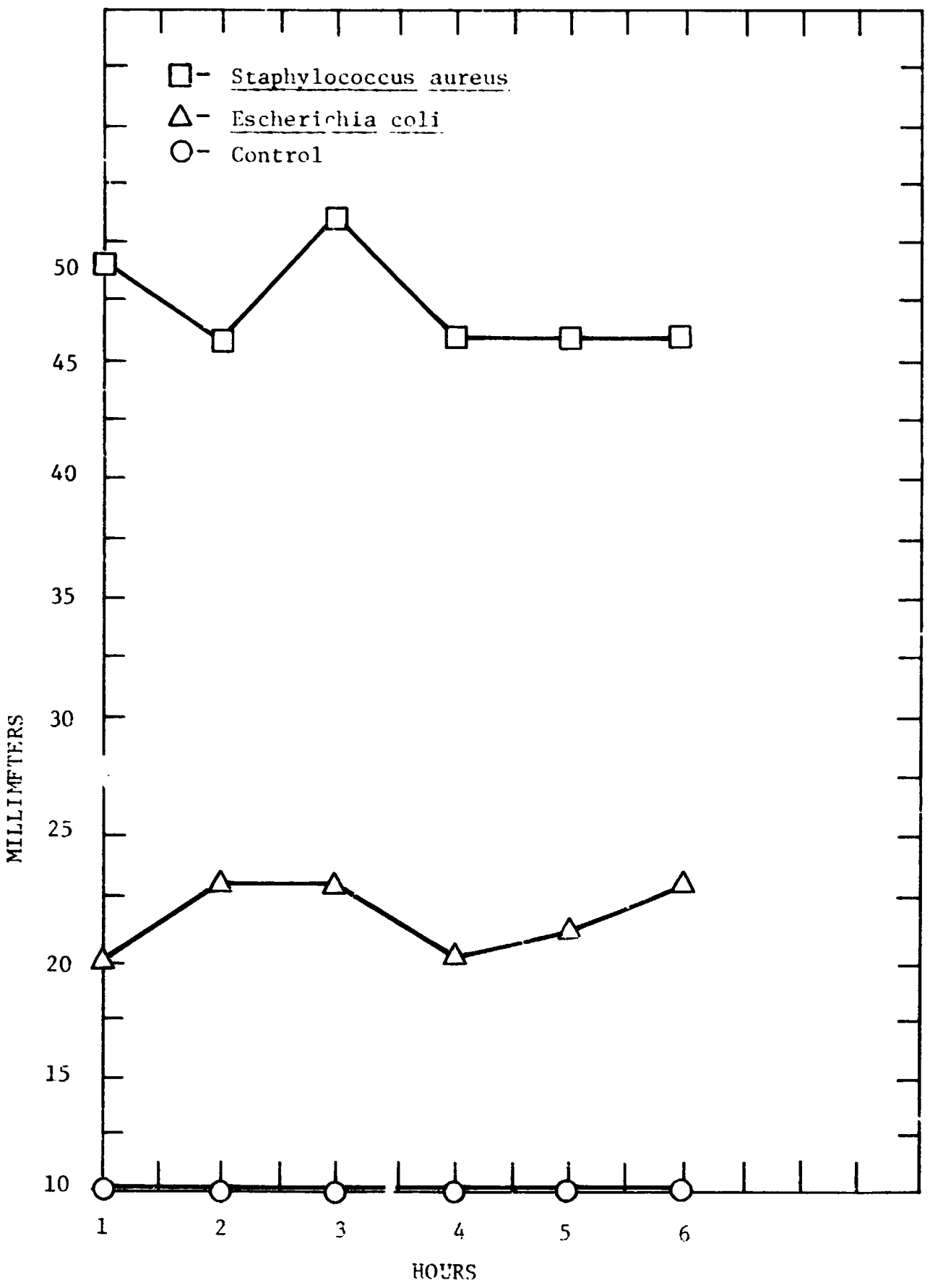


FIGURE 21: ZONES OF INHIBITION PRODUCED BY RTV 731 DISCS CONTAINING 5% PARAFORMALDEHYDE PREVIOUSLY HEATED FOR 1-6 HOURS AT 60C.

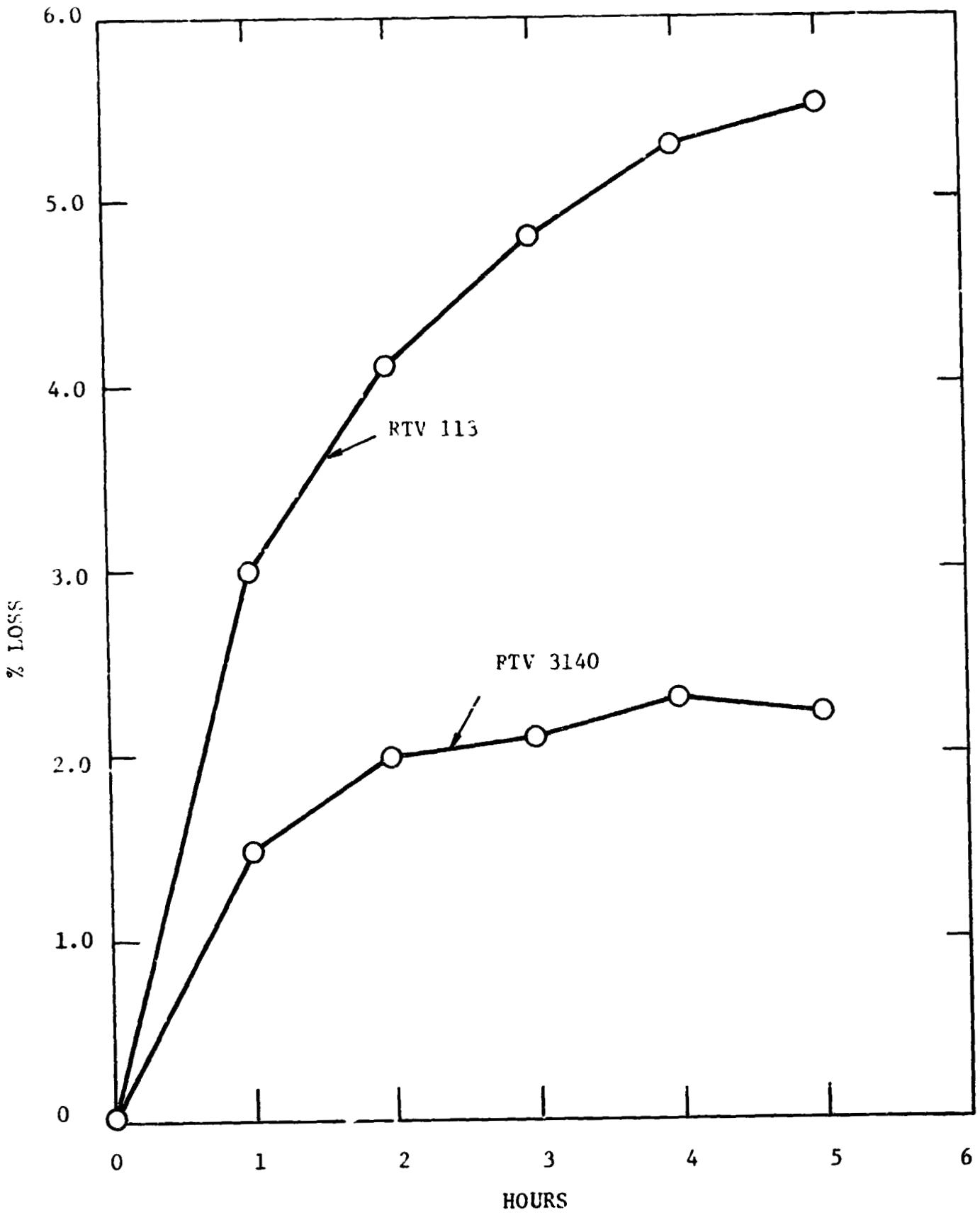


FIGURE 22: RELEASE OF FORMALDEHYDE FROM URAC 110 (10.6%) CONTAINED IN DISCS OF RTV DURING HEATING AT 60°C

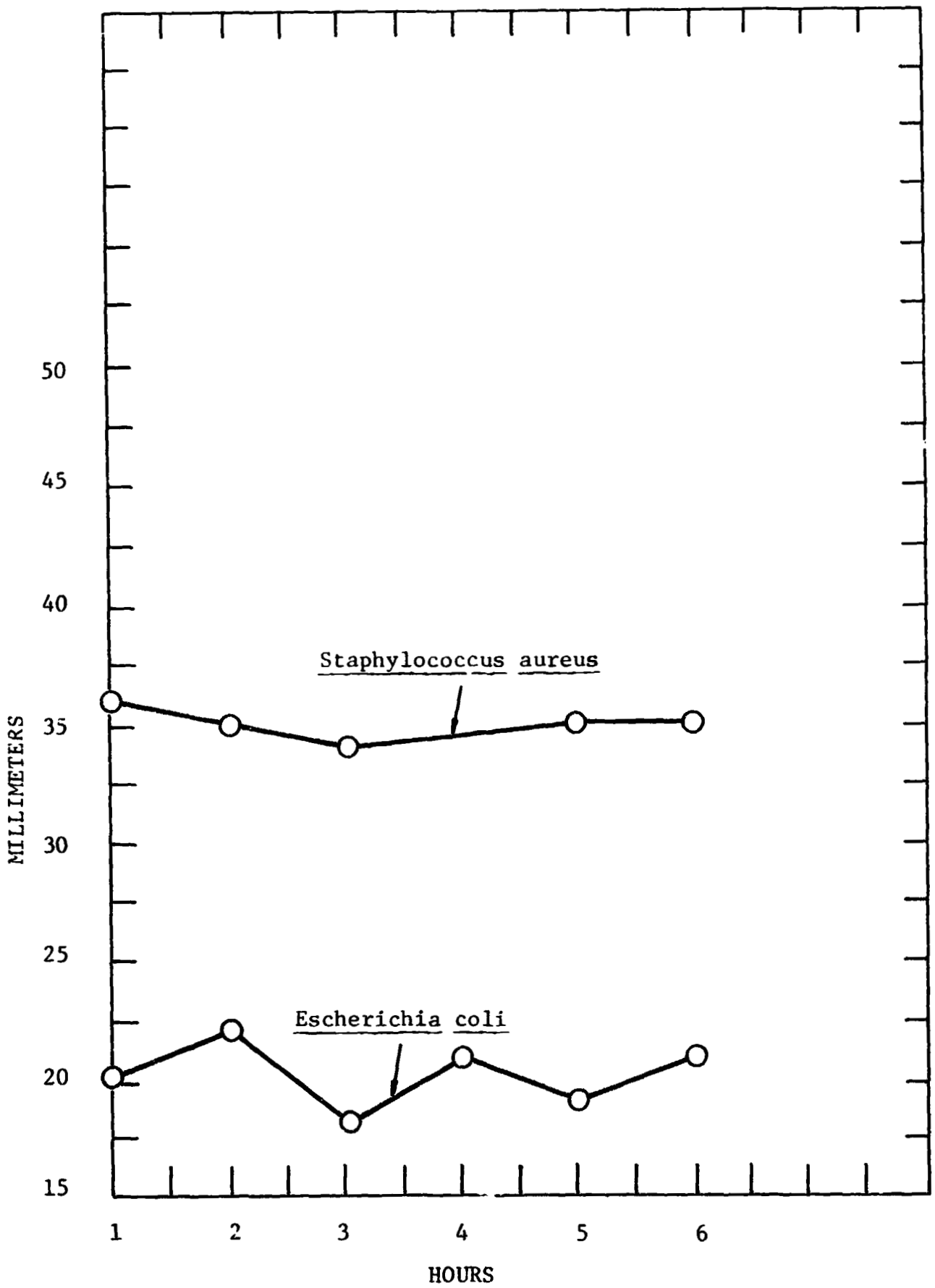


FIGURE 23: ZONES OF INHIBITION PRODUCED BY RTV 3140 DISCS CONTAINING 10.6% UREA FORMALDEHYDE PREVIOUSLY HEATED FOR 1-6 HOURS AT 60C.

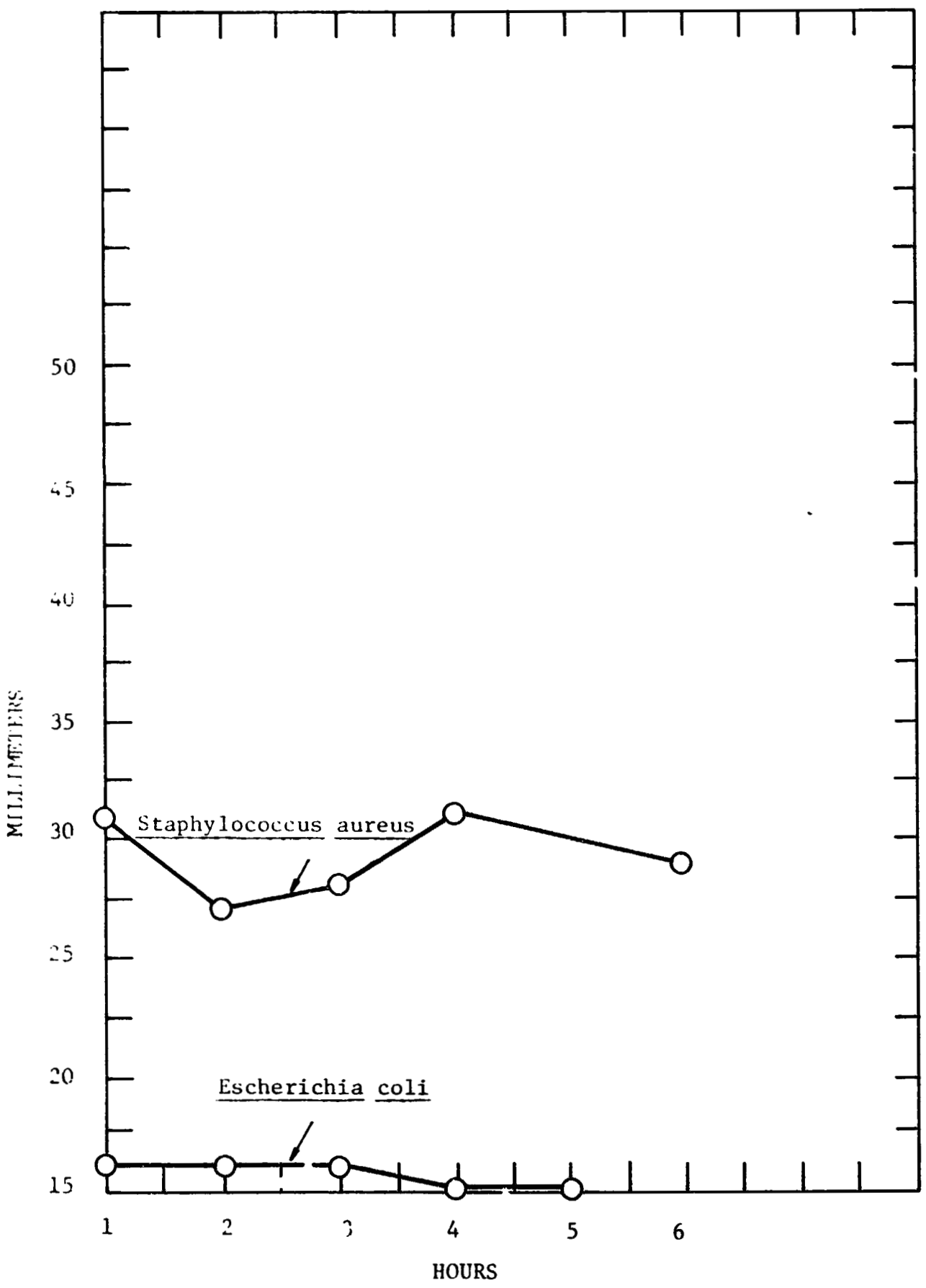


FIGURE 24: ZONES OF INHIBITION PRODUCED BY RTV 118 DISCS CONTAINING 10.6% UREA FORMALDEHYDE PREVIOUSLY HEATED FOR 1-6 HOURS AT 60C.

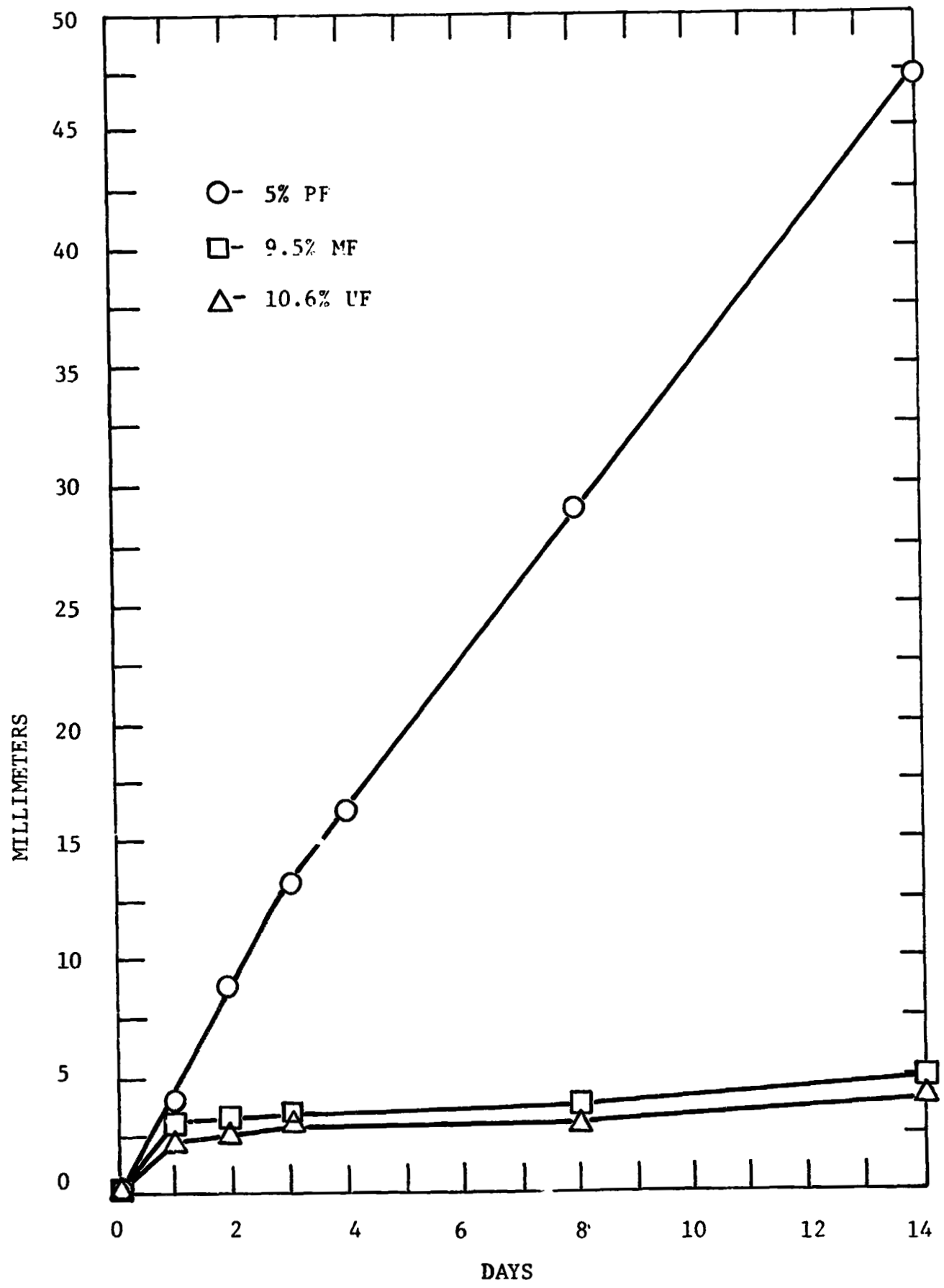


FIGURE 25: RELEASE OF FORMALDEHYDE FROM PARAFORMALDEHYDE (5%), MELAMINE FORMALDEHYDE (9.5%) AND UREA FORMALDEHYDE (10.6%) CONTAINED IN CHEM SEAL DISCS HEATED AT 60C.

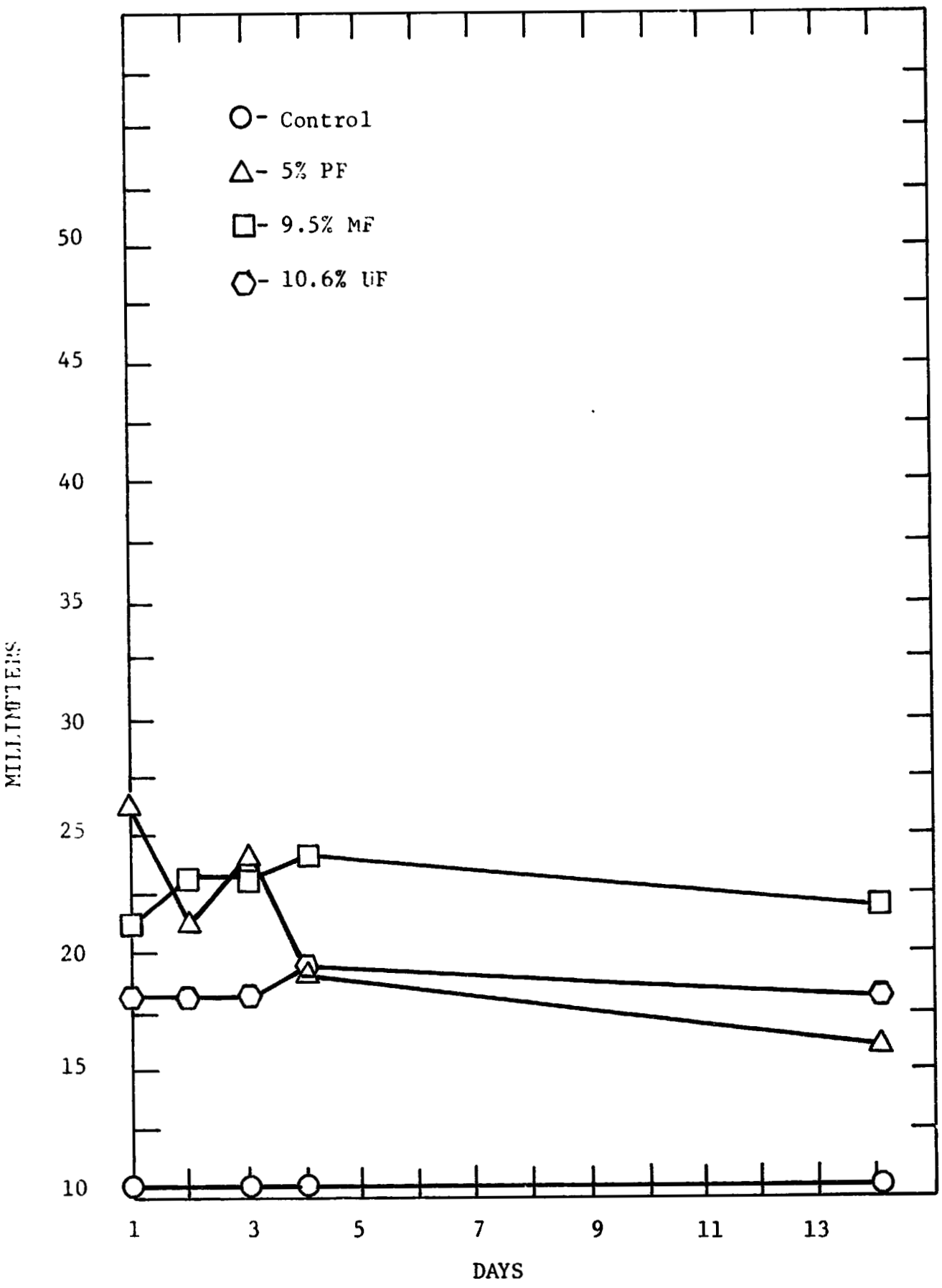


FIGURE 26: INHIBITION OF Staphylococcus aureus BY CHEM SEAL DISCS CONTAINING PARAFORMALDEHYDE (5%), MELAMINE FORMALDEHYDE (9.5%), AND UREA FORMALDEHYDE (10.6%) PREVIOUSLY HEATED AT 60C.

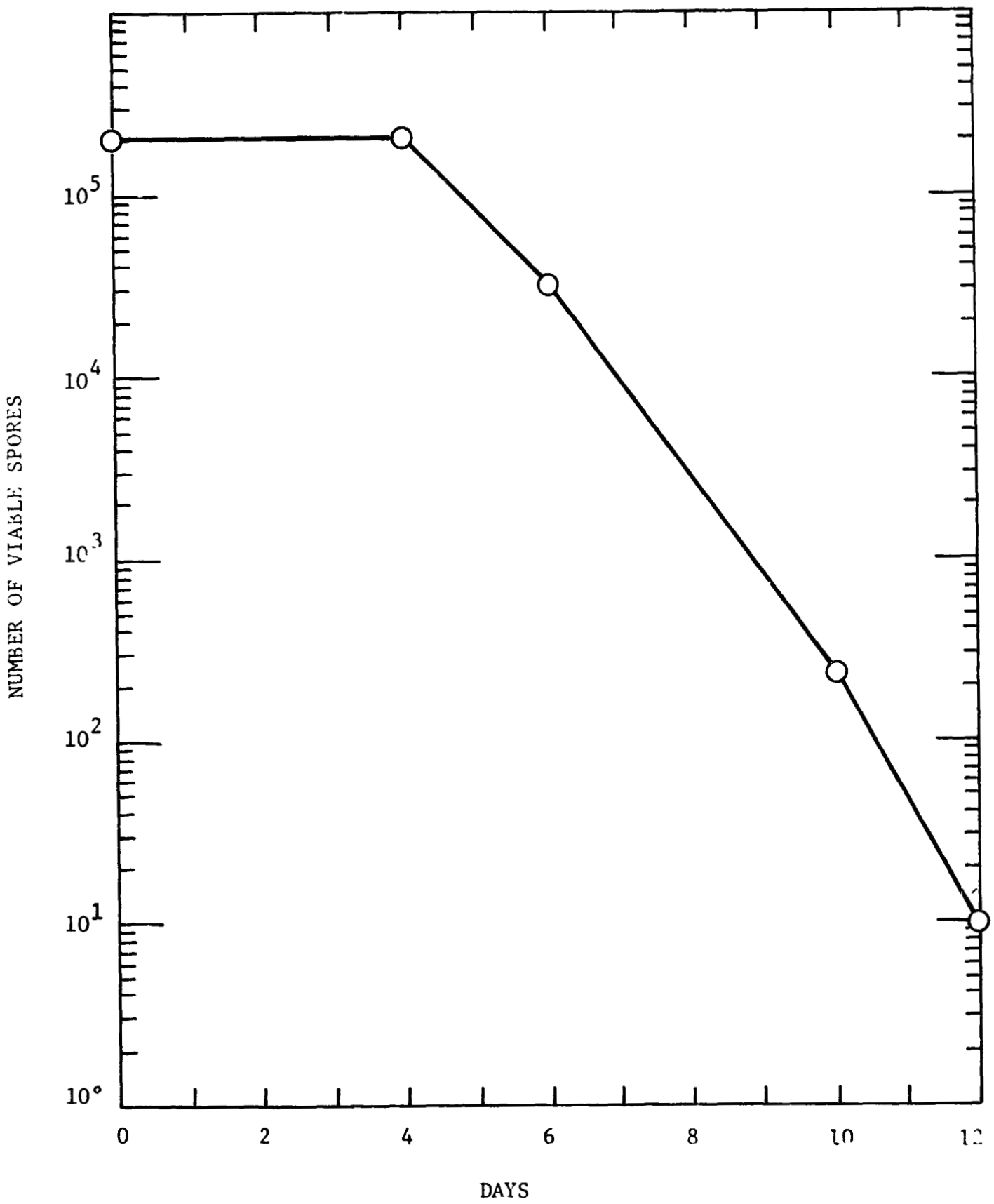


FIGURE 27: INACTIVATION OF Bacillus stearothermophilus SPORES ON STRIPS EMBEDDED IN RTV 3140 CONTAINING PARAFORMALDEHYDE (1%) DURING CURING AT 25 (±2) C.

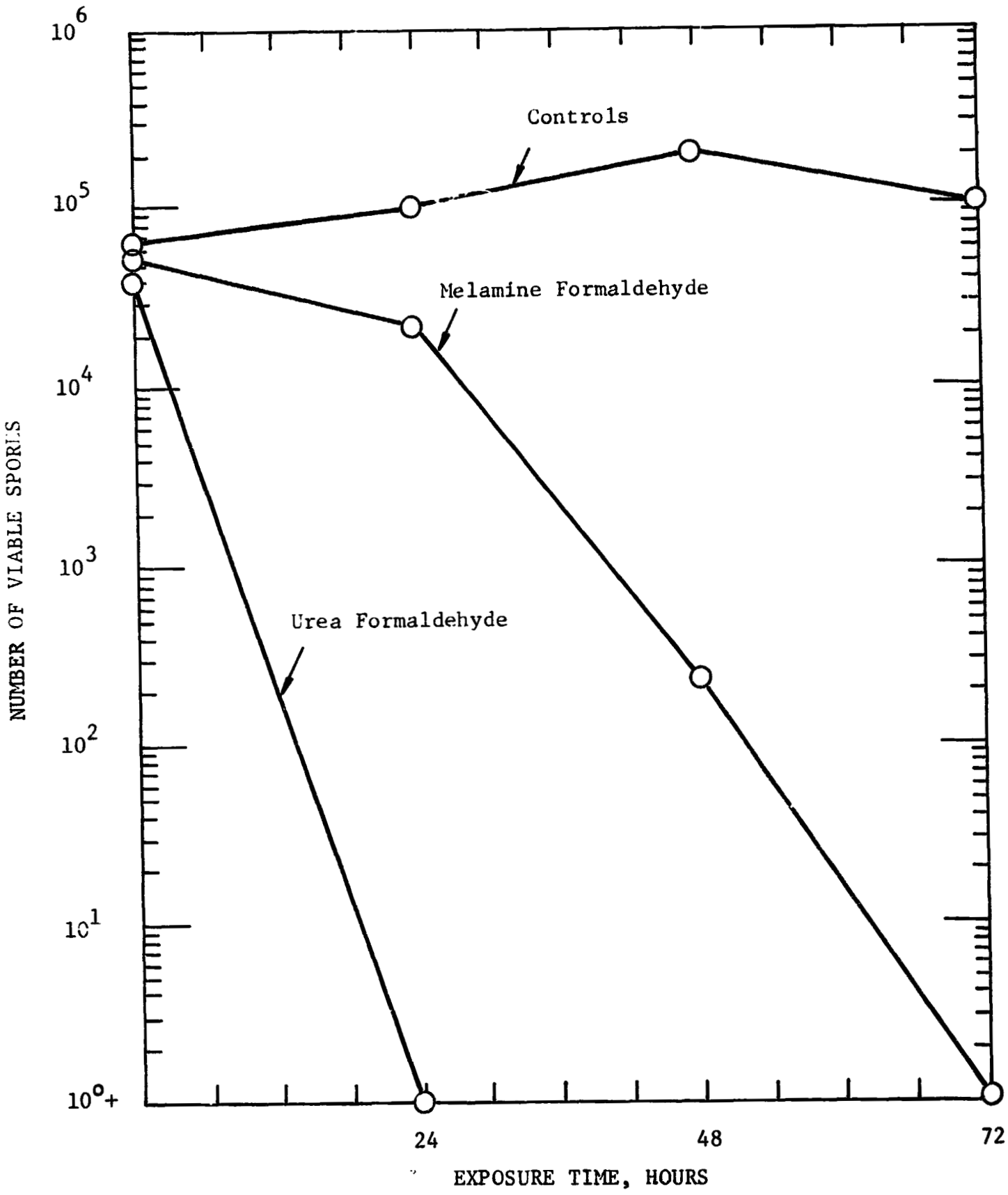


FIGURE 28: INACTIVATION OF Bacillus stearothermophilus SPORES ON STRIPS EMBEDDED IN RTV 3140 CONTAINING MELAMINE FORMALDEHYDE (1.9%) OR UREA FORMALDEHYDE (2.1%) DURING HEATING AT 60C.

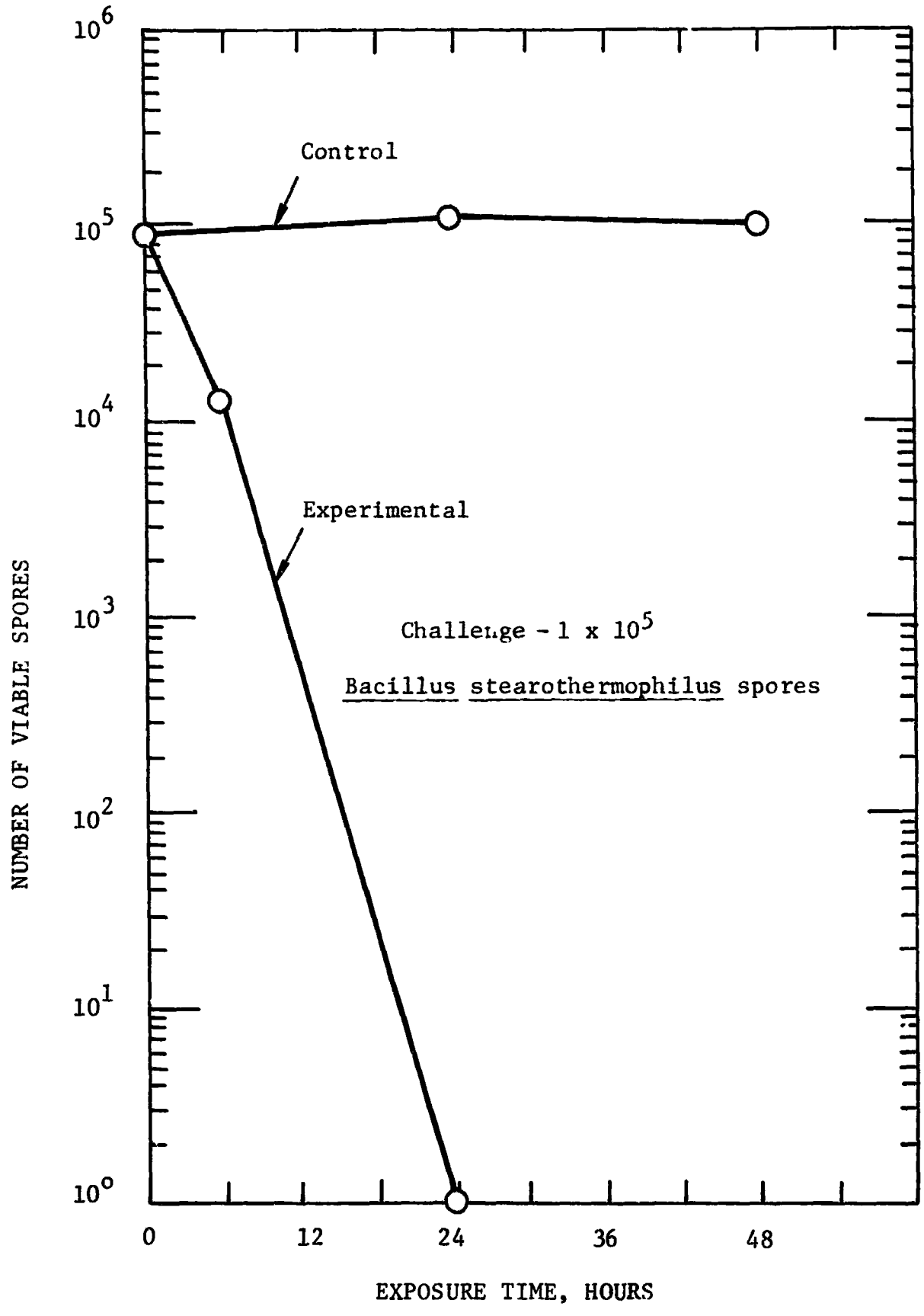


FIGURE 29: INTERNAL STERILIZATION OF RTV 3140 CONTAINING 0.1% PARAFORMALDEHYDE AT 60C.

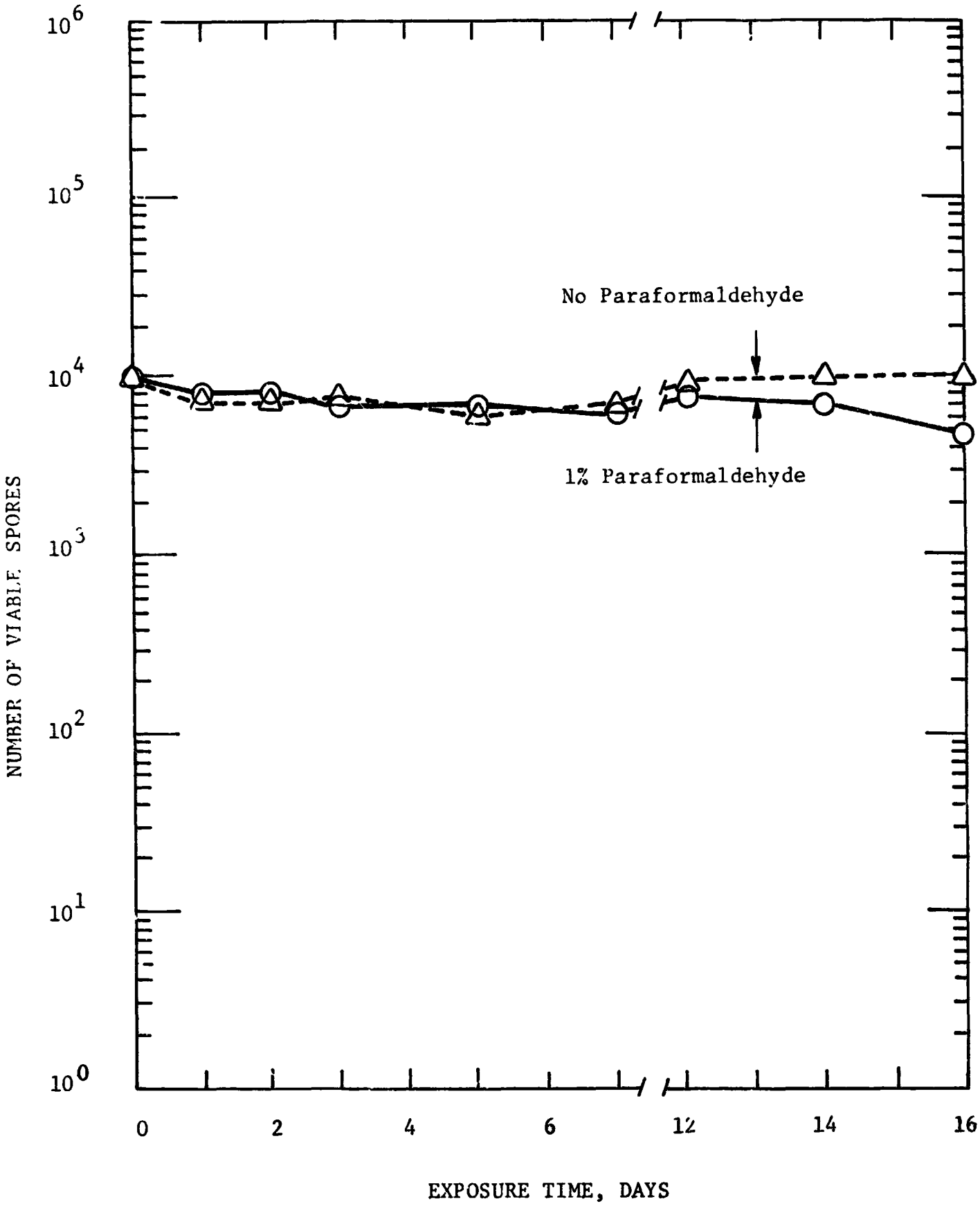


FIGURE 30: INACTIVATION OF Bacillus stearothermophilus SPORES IN CHEM SEAL 3457 CONTAINING 1% PARAFORMALDEHYDE AT 25C

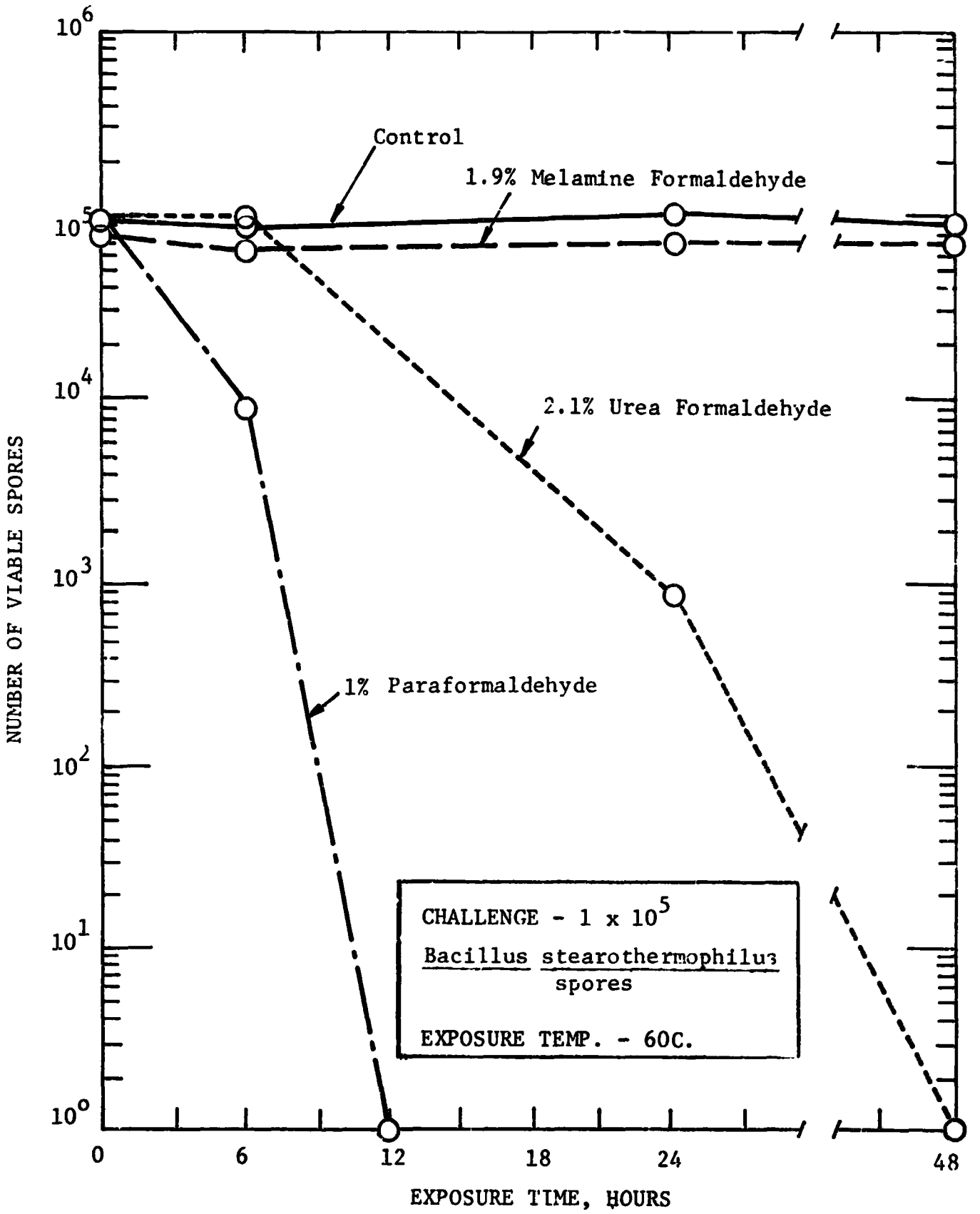


FIGURE 31: INTERNAL STERILIZATION OF CHEM SEAL 3547 CONTAINING VARIOUS STERILANT ADDITIVES.

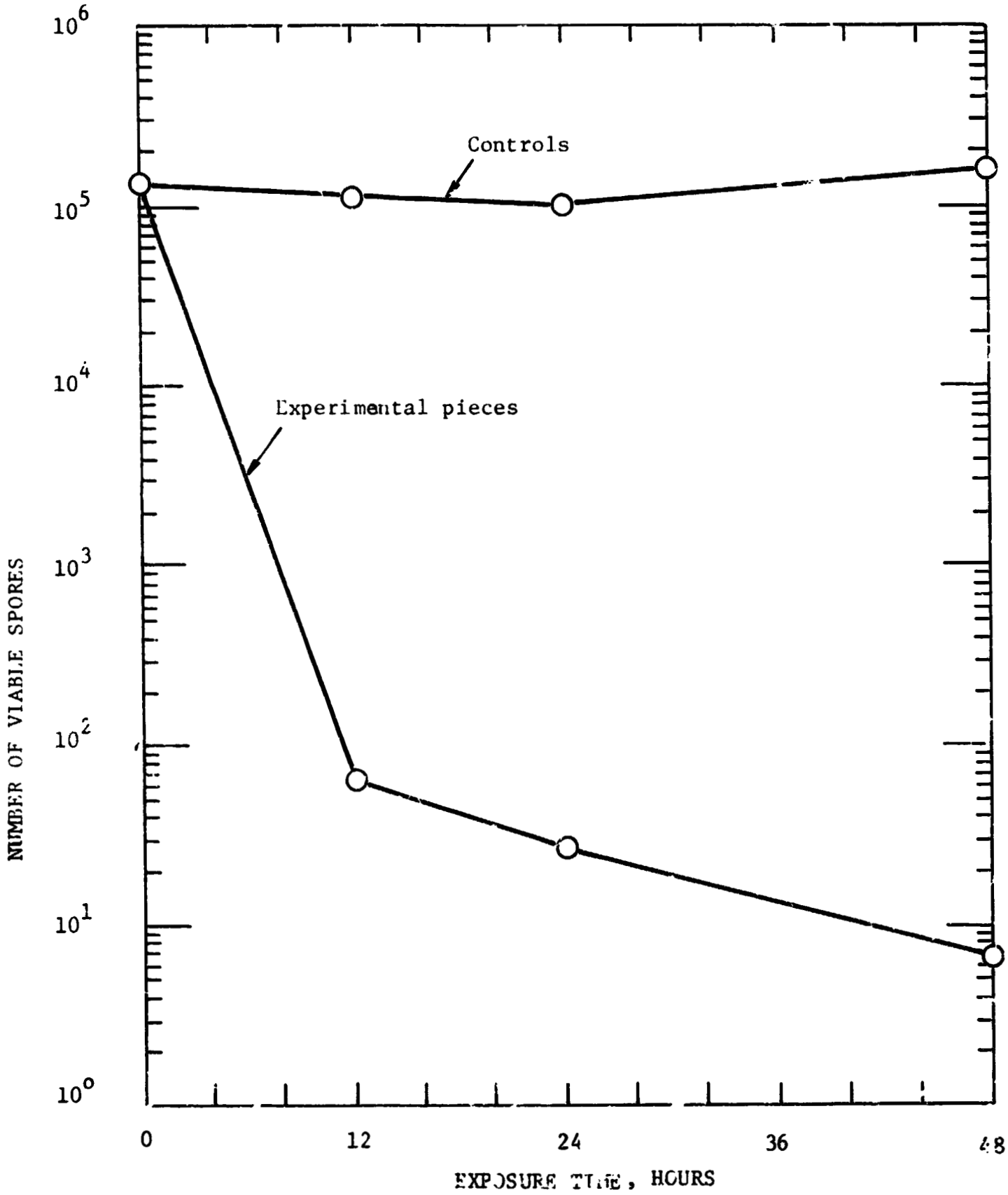


FIGURE 32: INACTIVATION OF Bacillus stearothermophilus SPORES ON OCCLUDED SURFACES (THREADS) OF SPACECRAFT TUBING CONNECTORS EXPOSED TO 1% PARAFORMALDEHYDE IN TRICHLOROETHYLENE AT 60C.

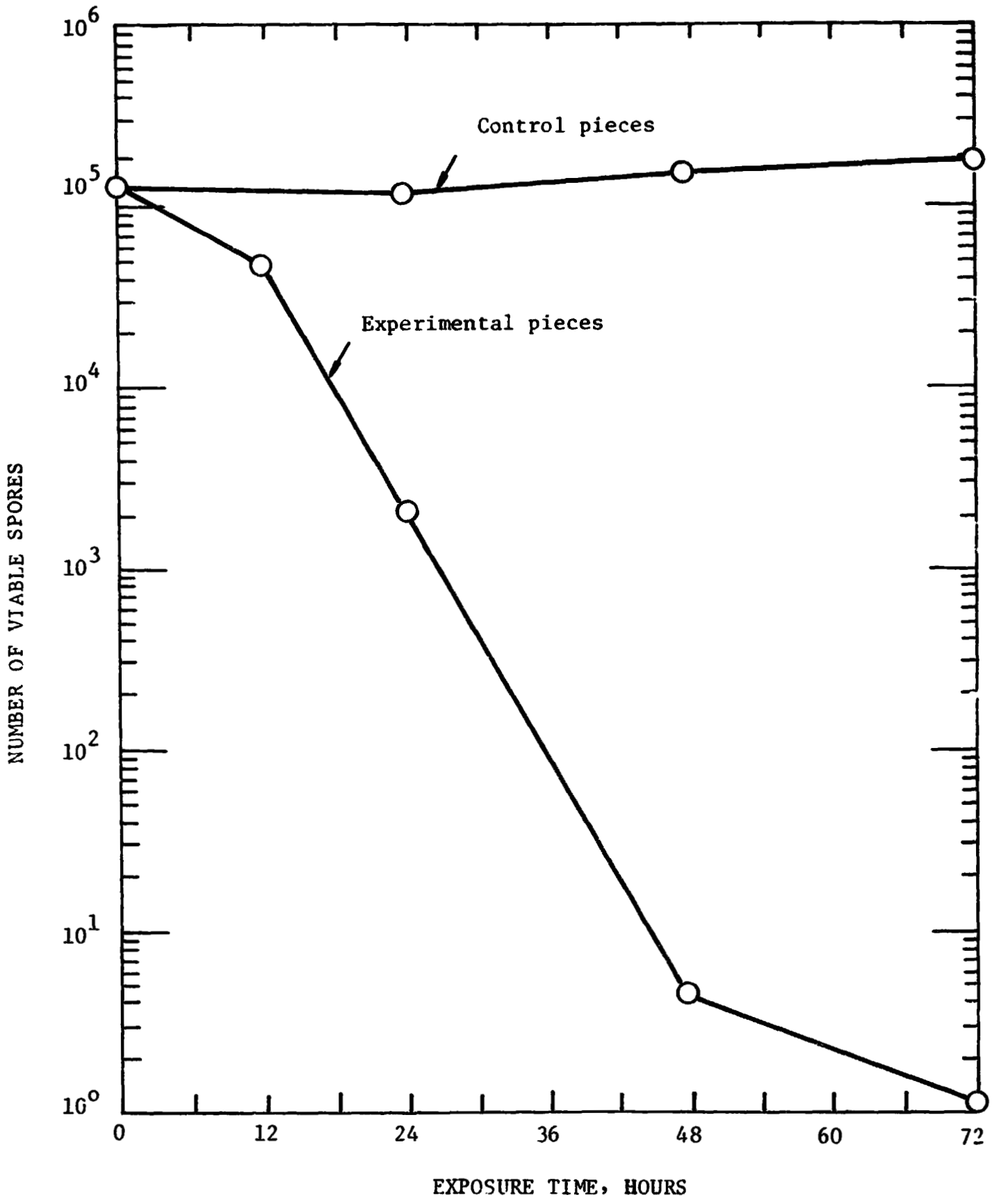


FIGURE 33: INACTIVATION OF Bacillus stearothermophilus SPORES ON OCCLUDED AREAS OF SPACECRAFT HARDWARE WITH 0.1% PARAFORMALDEHYDE-TRICHLOROETHYLENE SPRAY.

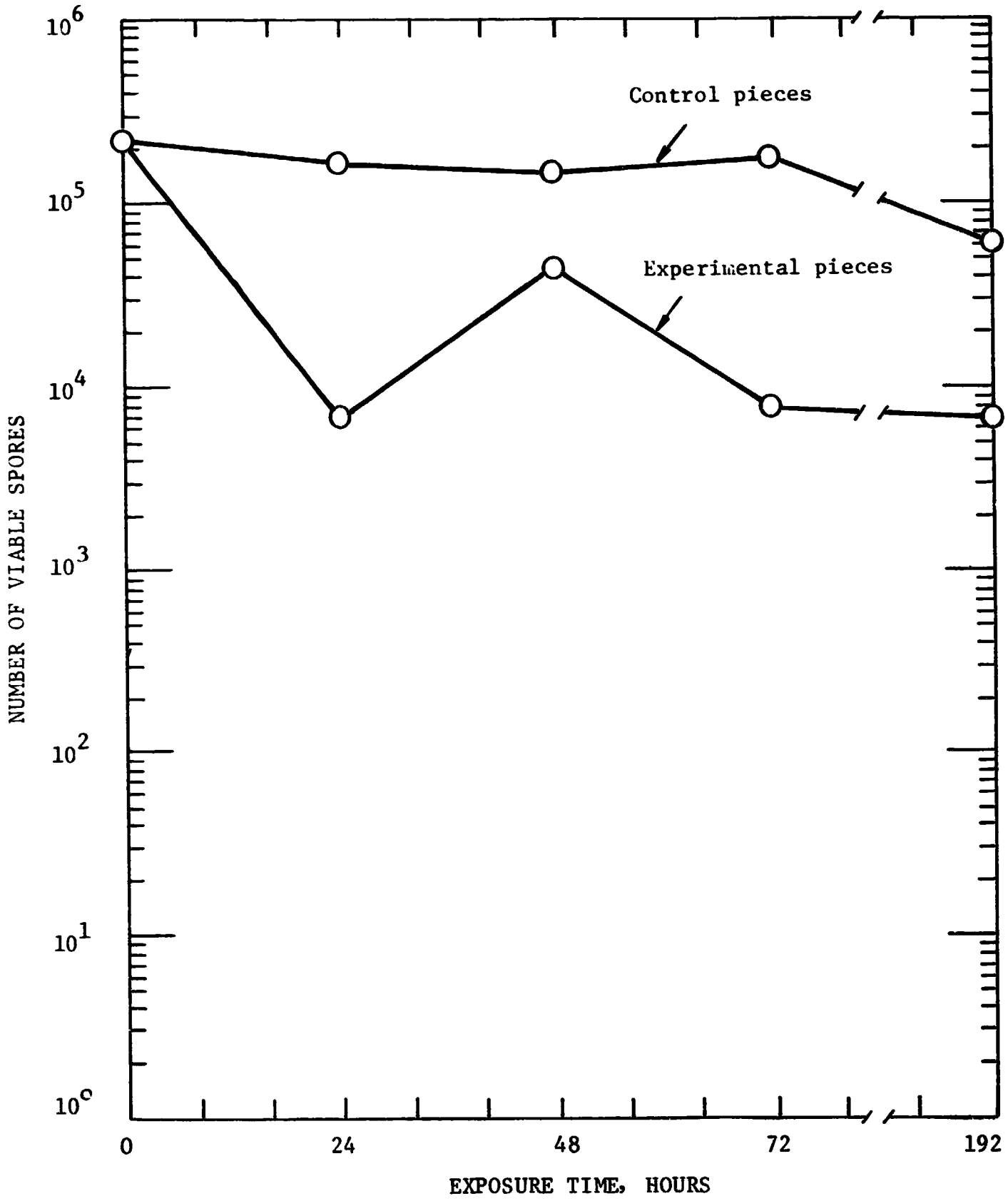


FIGURE 34: INACTIVATION OF Bacillus stearothermophilus SPORES ON MATED SURFACES OF SPACECRAFT TUBING CONNECTORS (STAINLESS STEEL) AT 60C WITH 1.0% PARAFORMALDEHYDE - TRICHLOROETHYLENE SPRAY.

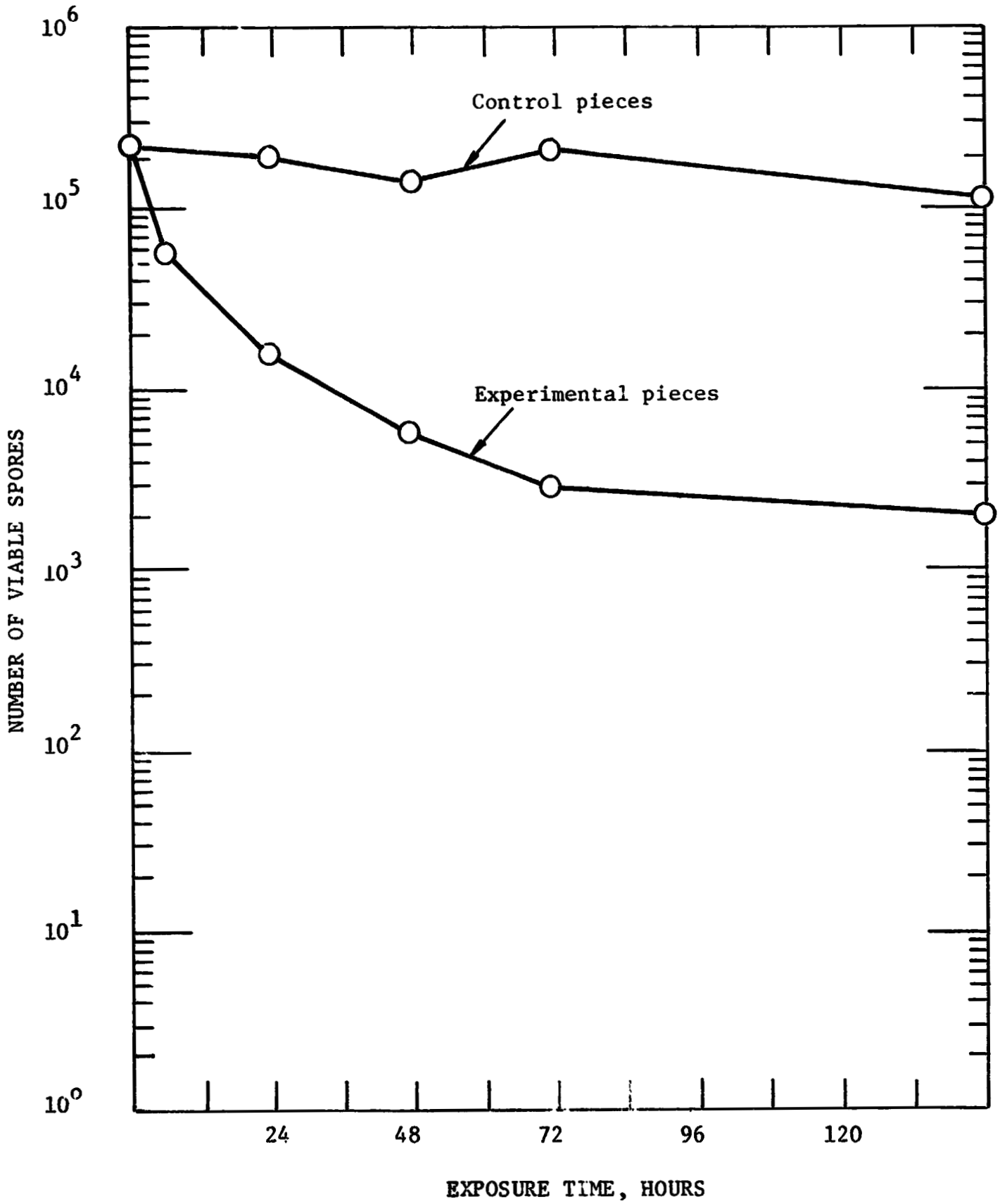


FIGURE 35: INACTIVATION OF Bacillus stearothermophilus SPORES ON MATED SURFACES OF SPACECRAFT TUBING CONNECTORS (STAINLESS STEEL) AT 60C WITH 0.1% PARAFORMALDEHYDE - TRICHLOROETHYLENE SPRAY.

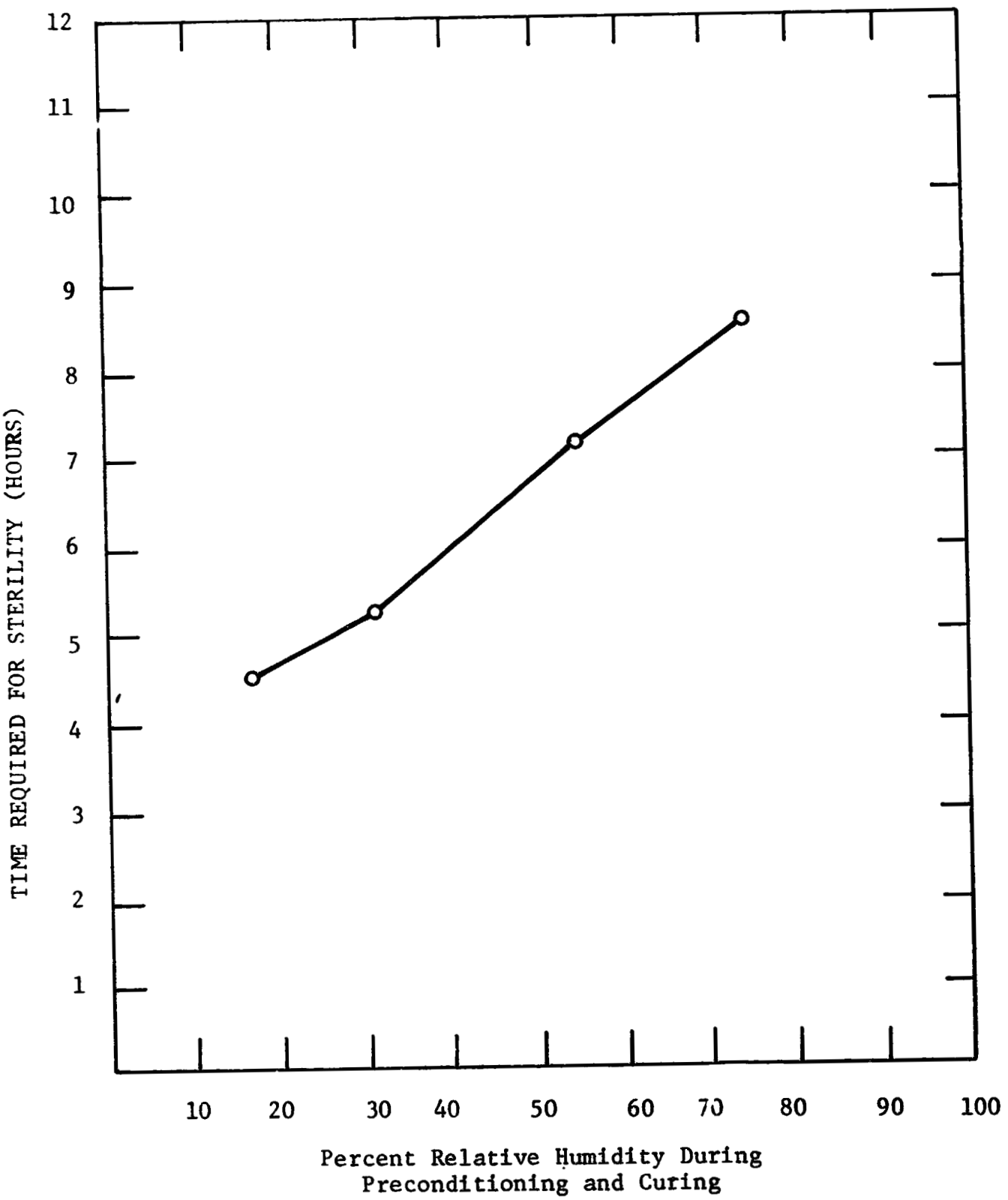


FIGURE 36: EFFECT OF MOISTURE ON FORMALDEHYDE STERILIZATION OF SPORES EMBEDDED IN RTV 3140

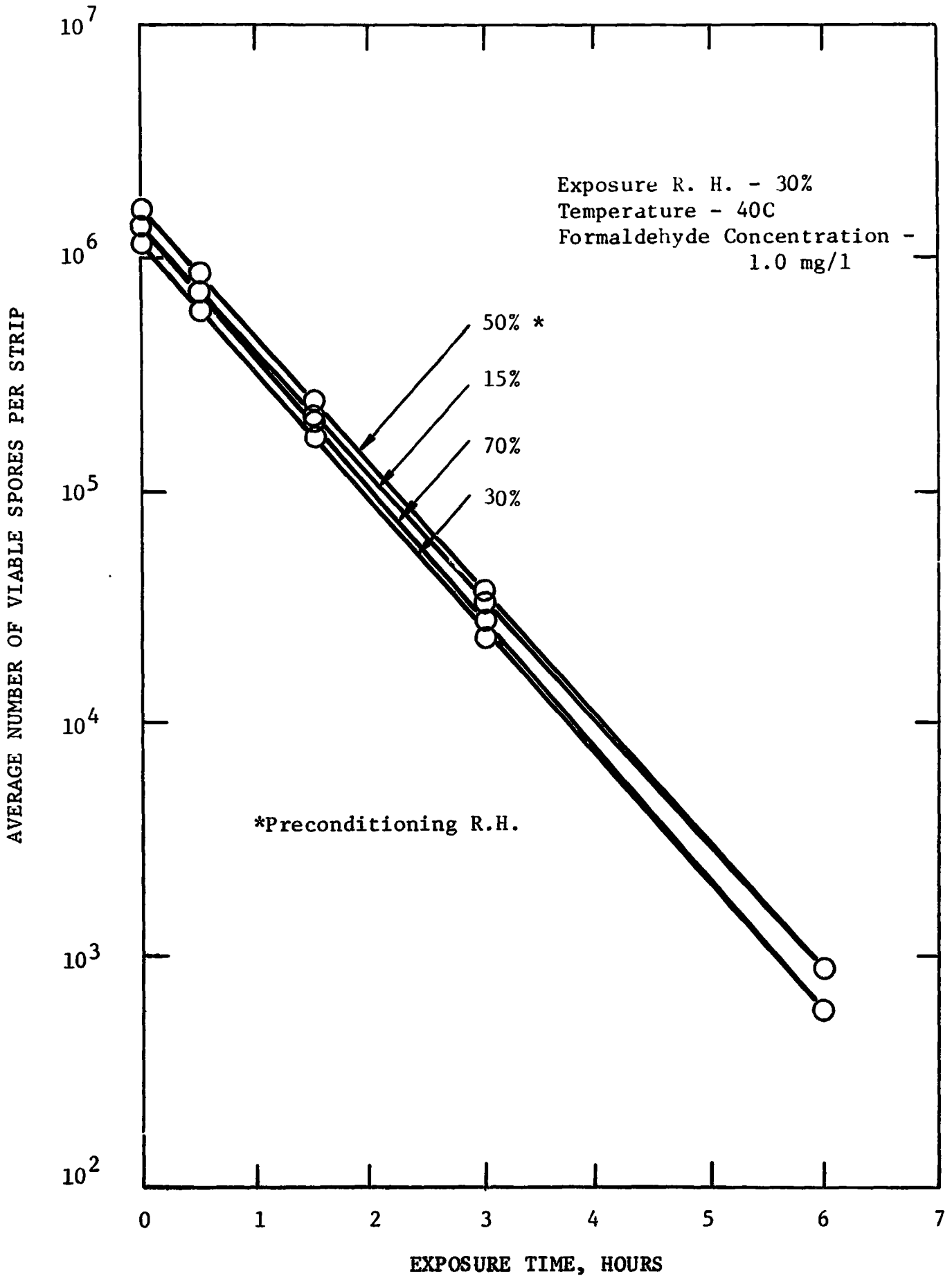


FIGURE 37: INACTIVATION OF Bacillus subtilis var. niger SPORES ON PAPER STRIPS AT 30% EXPOSURE R.H.

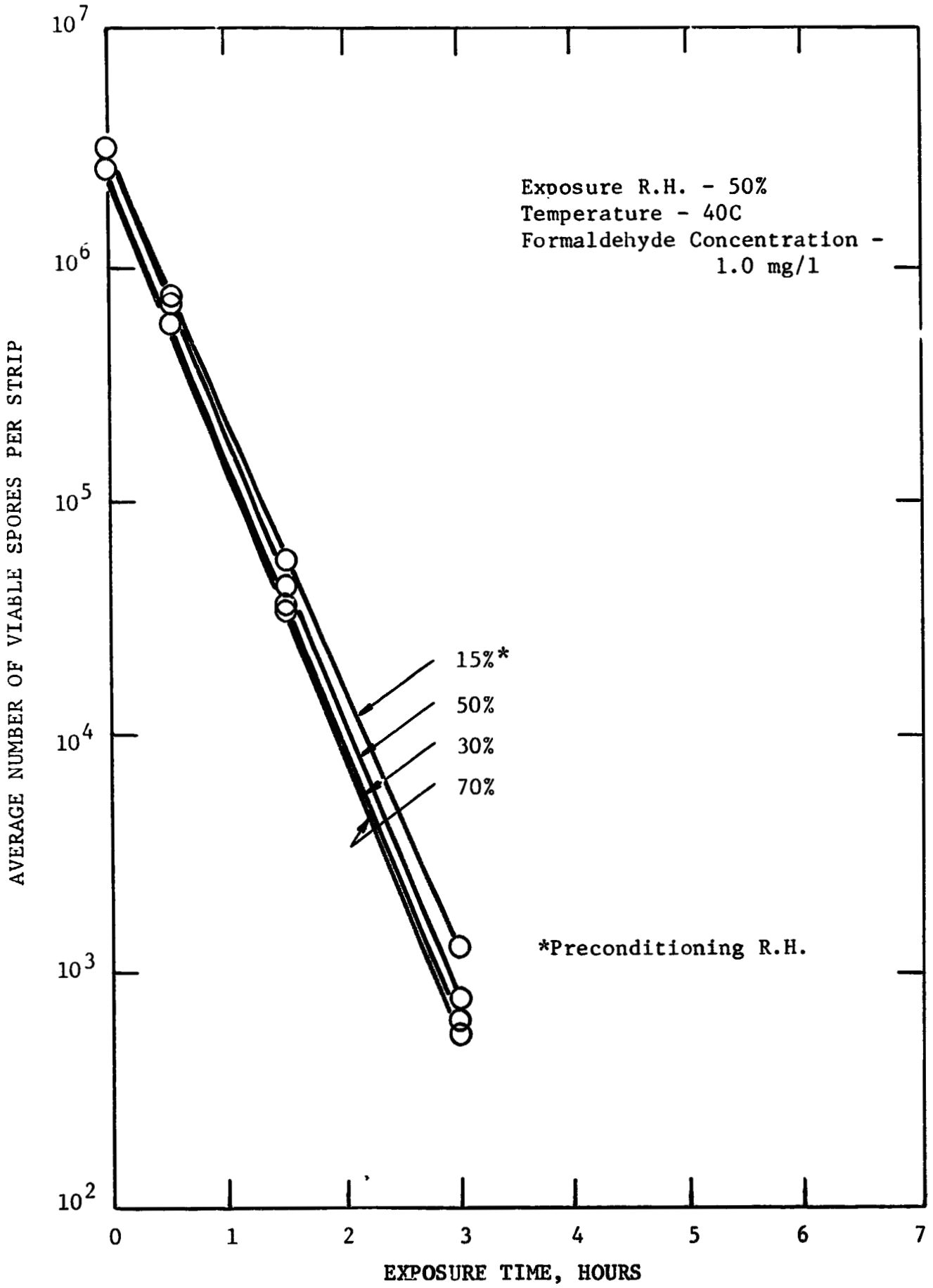


FIGURE 38: INACTIVATION OF Bacillus subtilis var. niger SPORES ON PAPER STRIPS AT 50% EXPOSURE R.H.

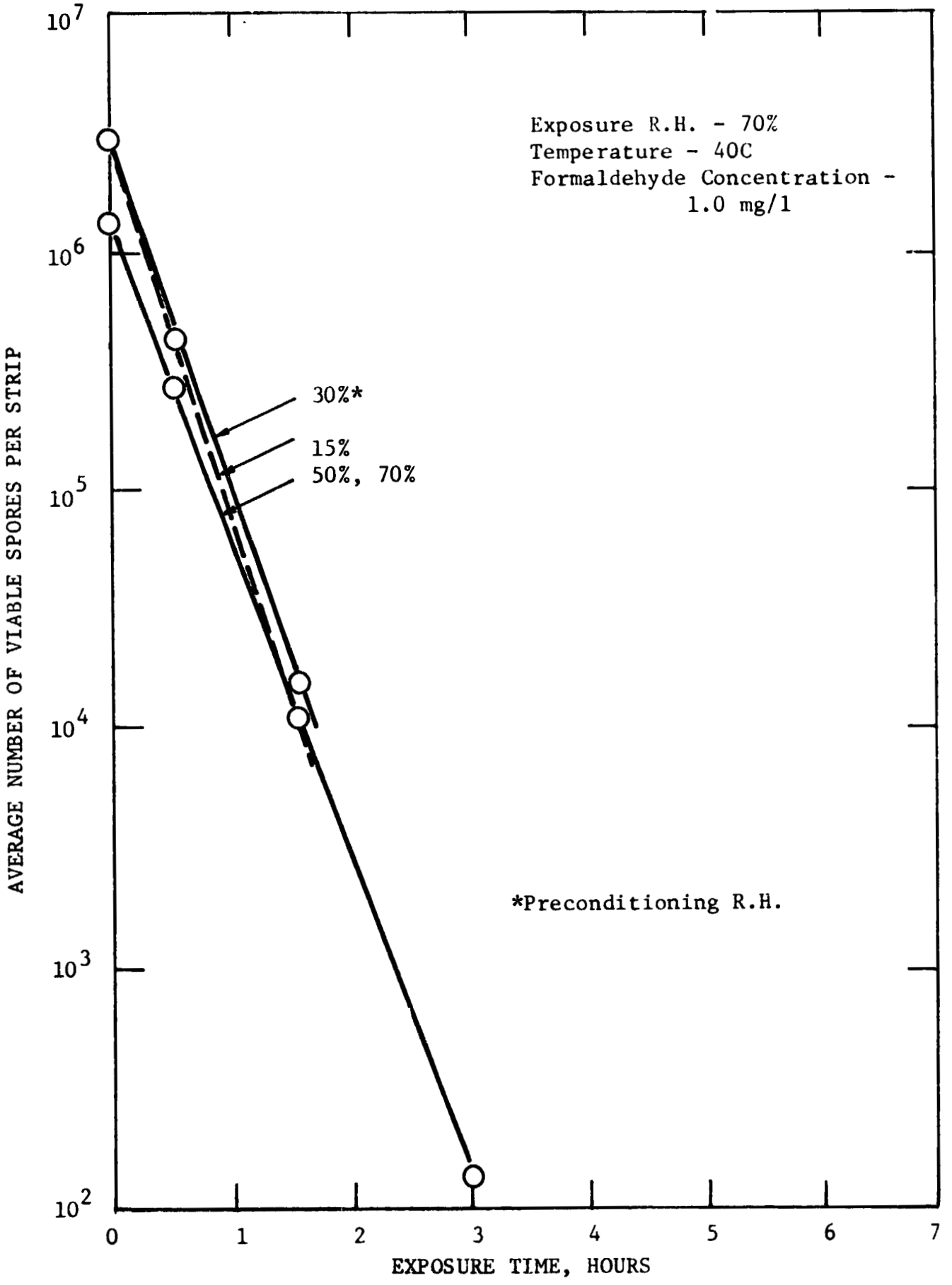


FIGURE 39: INACTIVATION OF Bacillus subtilis var. niger SPORES ON PAPER STRIPS AT 70% EXPOSURE R.H.

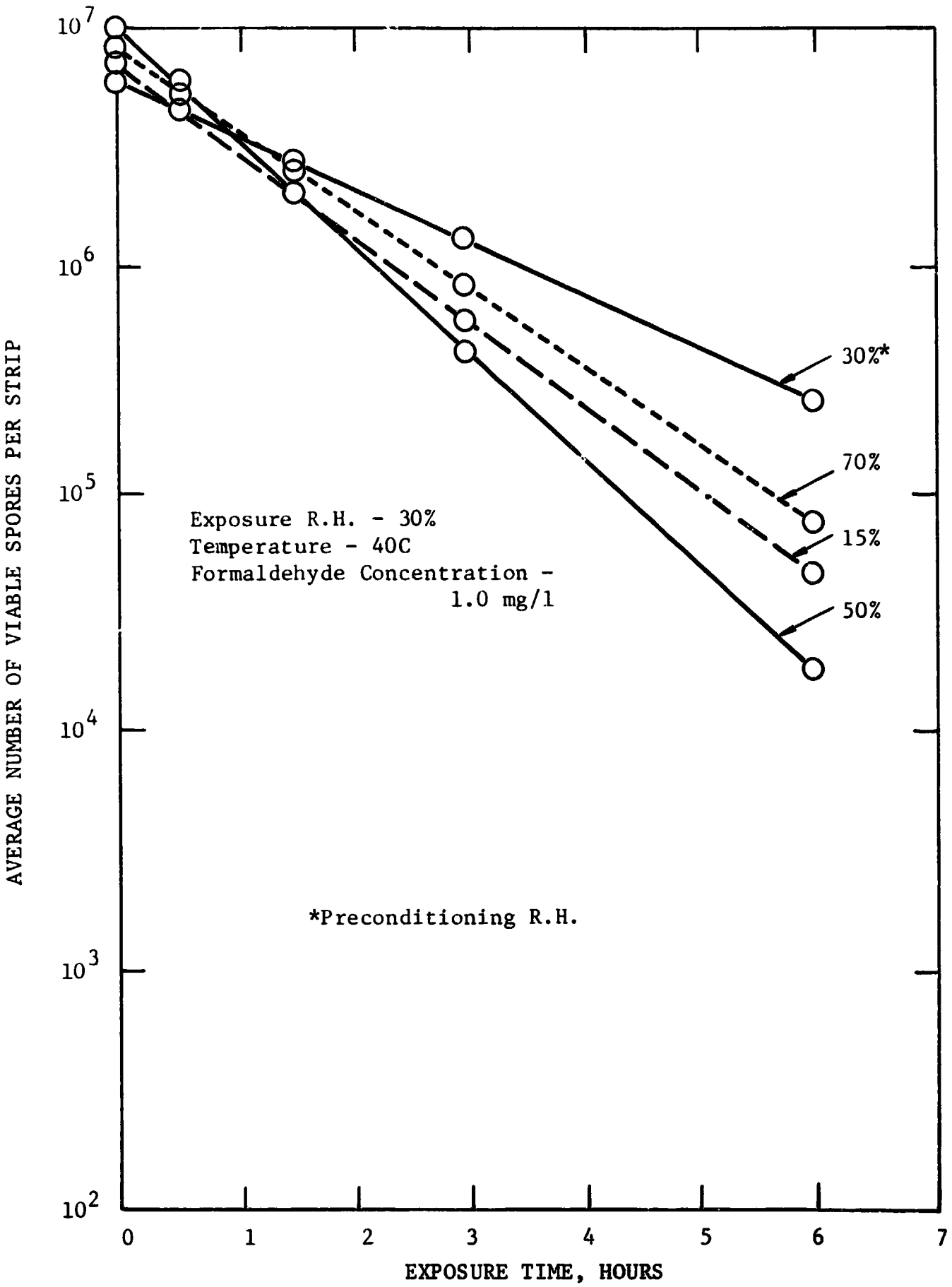


FIGURE 40: INACTIVATION OF Bacillus subtilis var. niger SPORES ON STAINLESS STEEL STRIPS AT 30% EXPOSURE R.H.

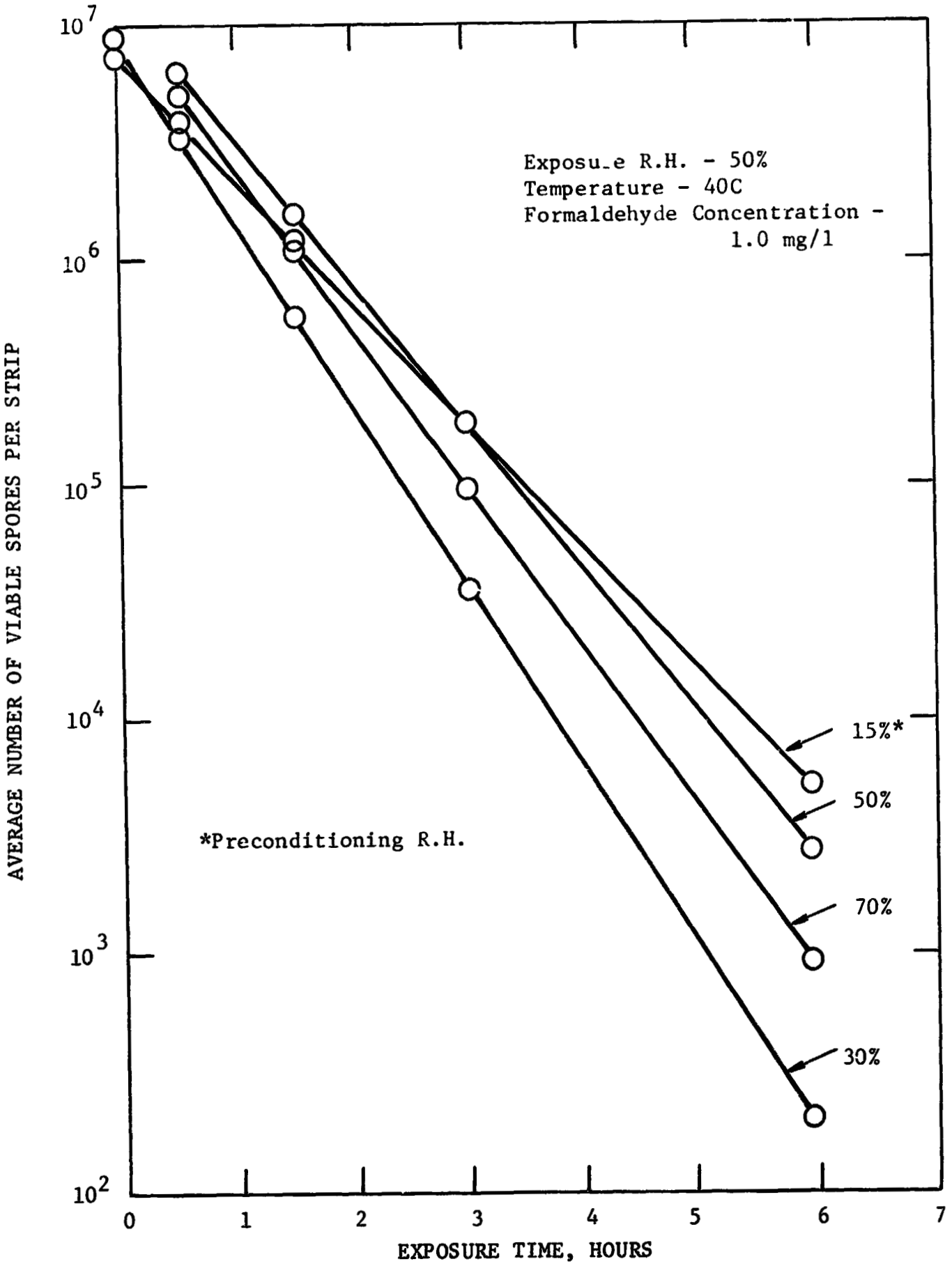


FIGURE 41: INACTIVATION OF Bacillus subtilis var. niger SPORES ON STAINLESS STEEL AT 50% EXPOSURE R.H.

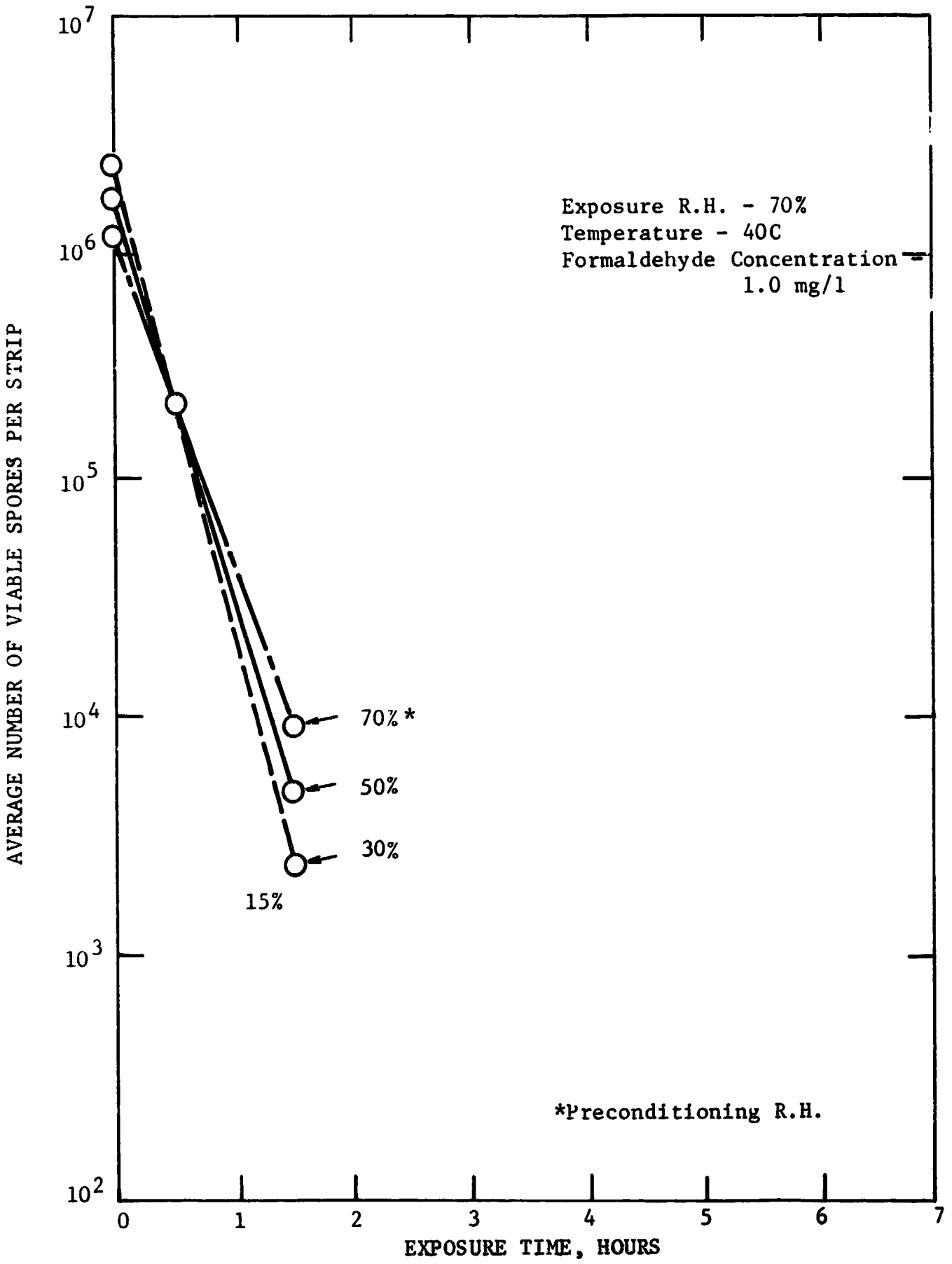


FIGURE 42: INACTIVATION OF Bacillus subtilis var. niger SPORES ON STAINLESS STEEL AT 70% EXPOSURE R.H.

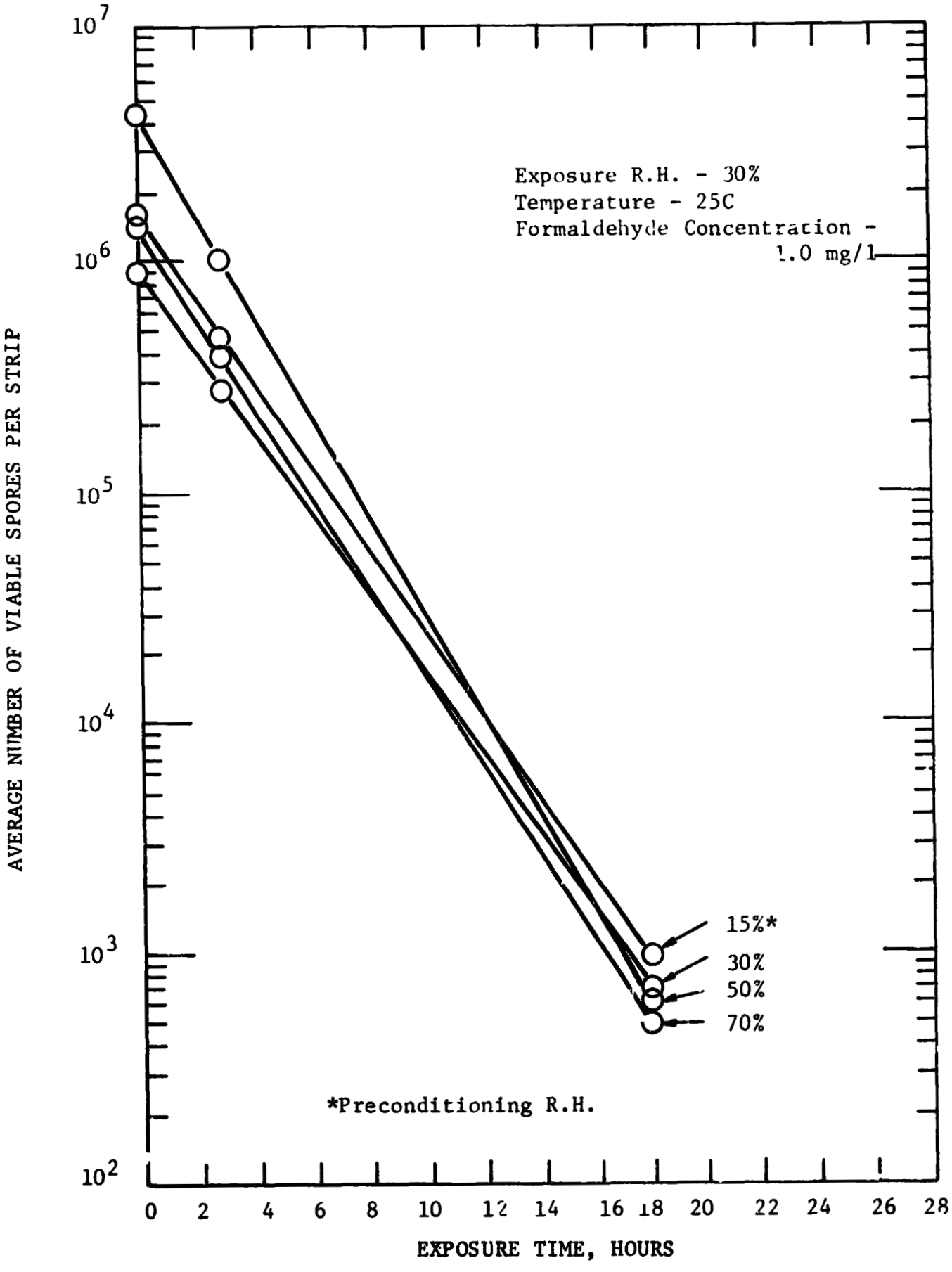


FIGURE 43: INACTIVATION OF Bacillus subtilis var. niger SPORES ON FILTER PAPER STRIPS

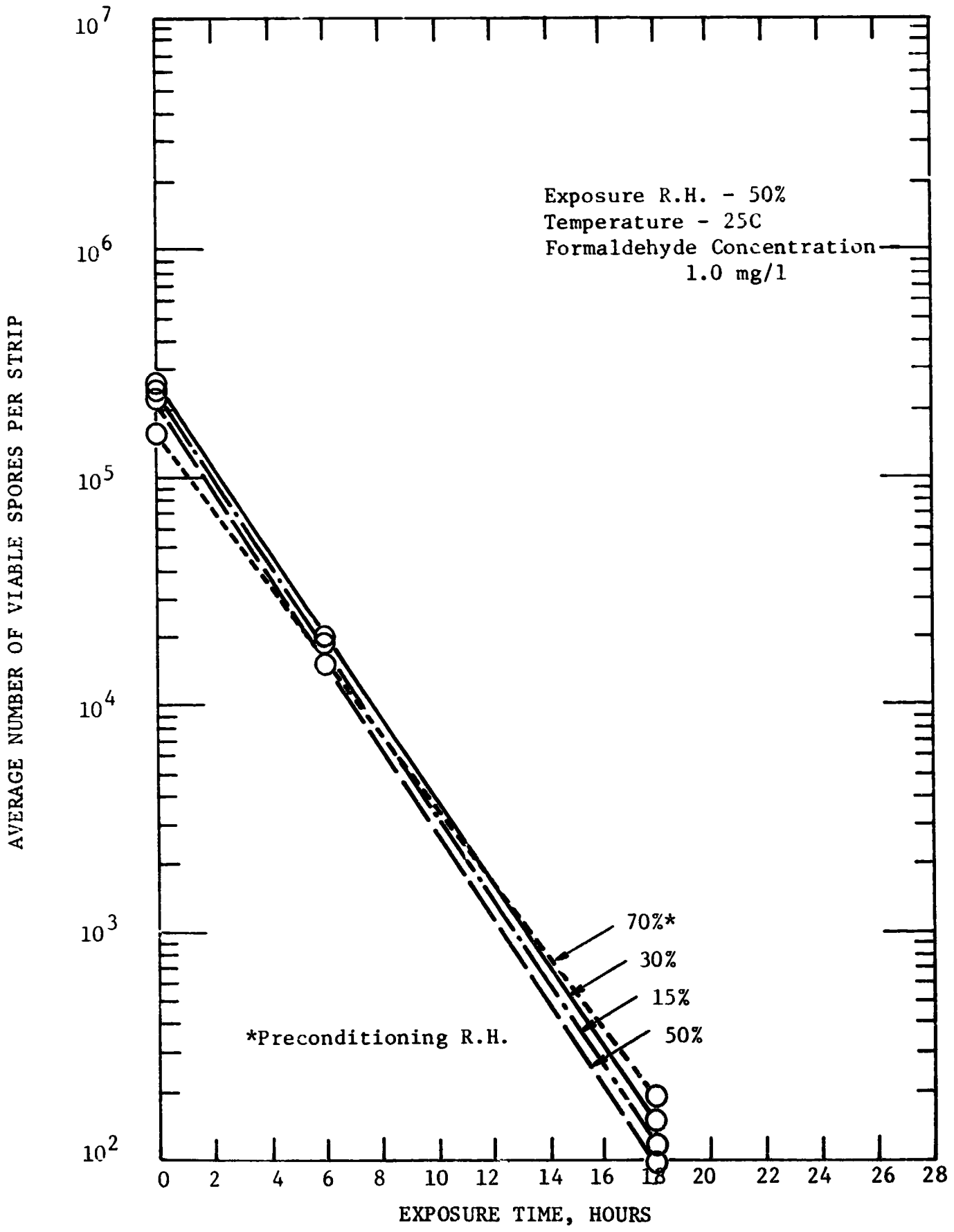


FIGURE 44: INACTIVATION OF Bacillus subtilis var. niger SPORES ON FILTER PAPER STRIPS

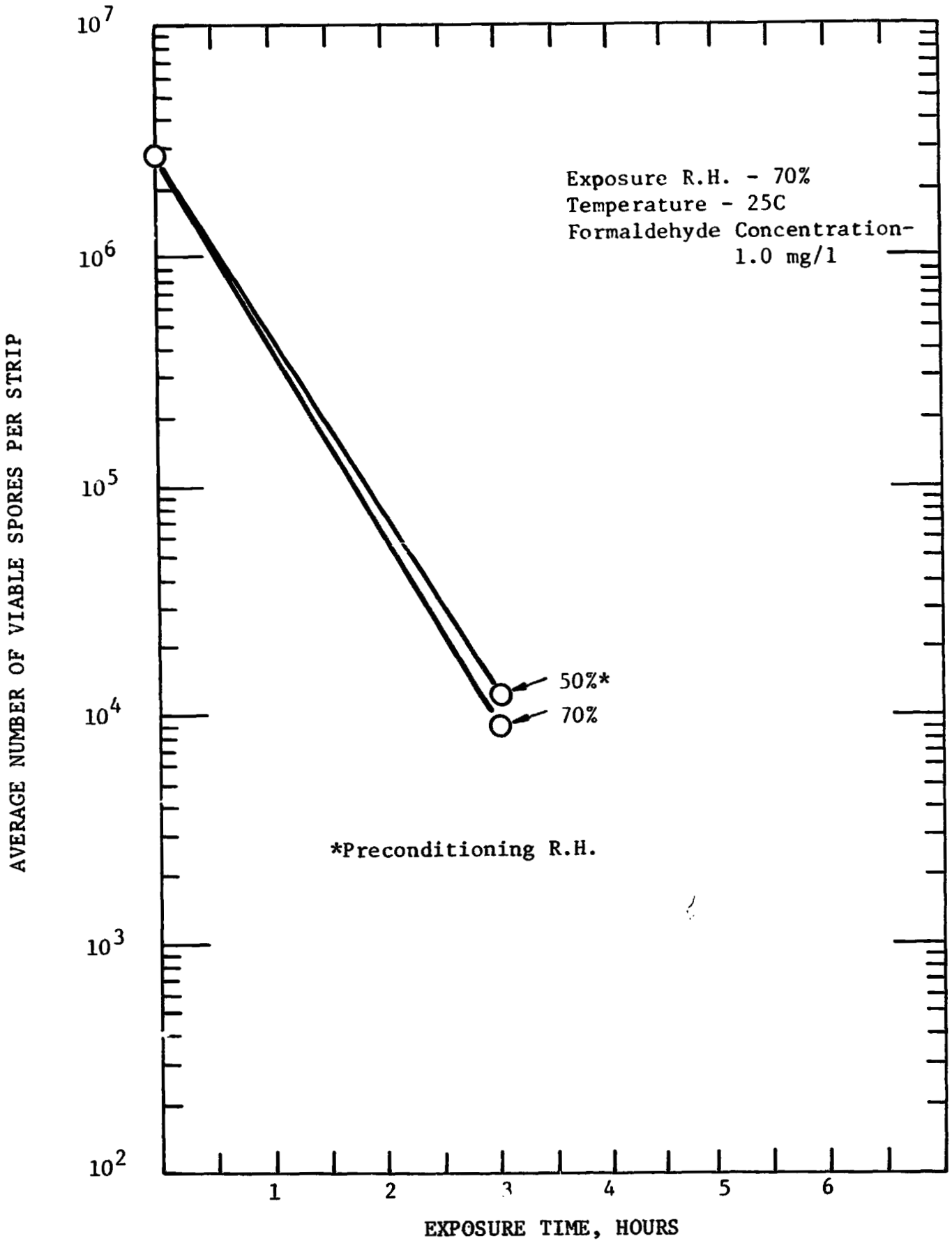


FIGURE 45: INACTIVATION OF Bacillus subtilis var. niger SPORES ON FILTER PAPER STRIPS

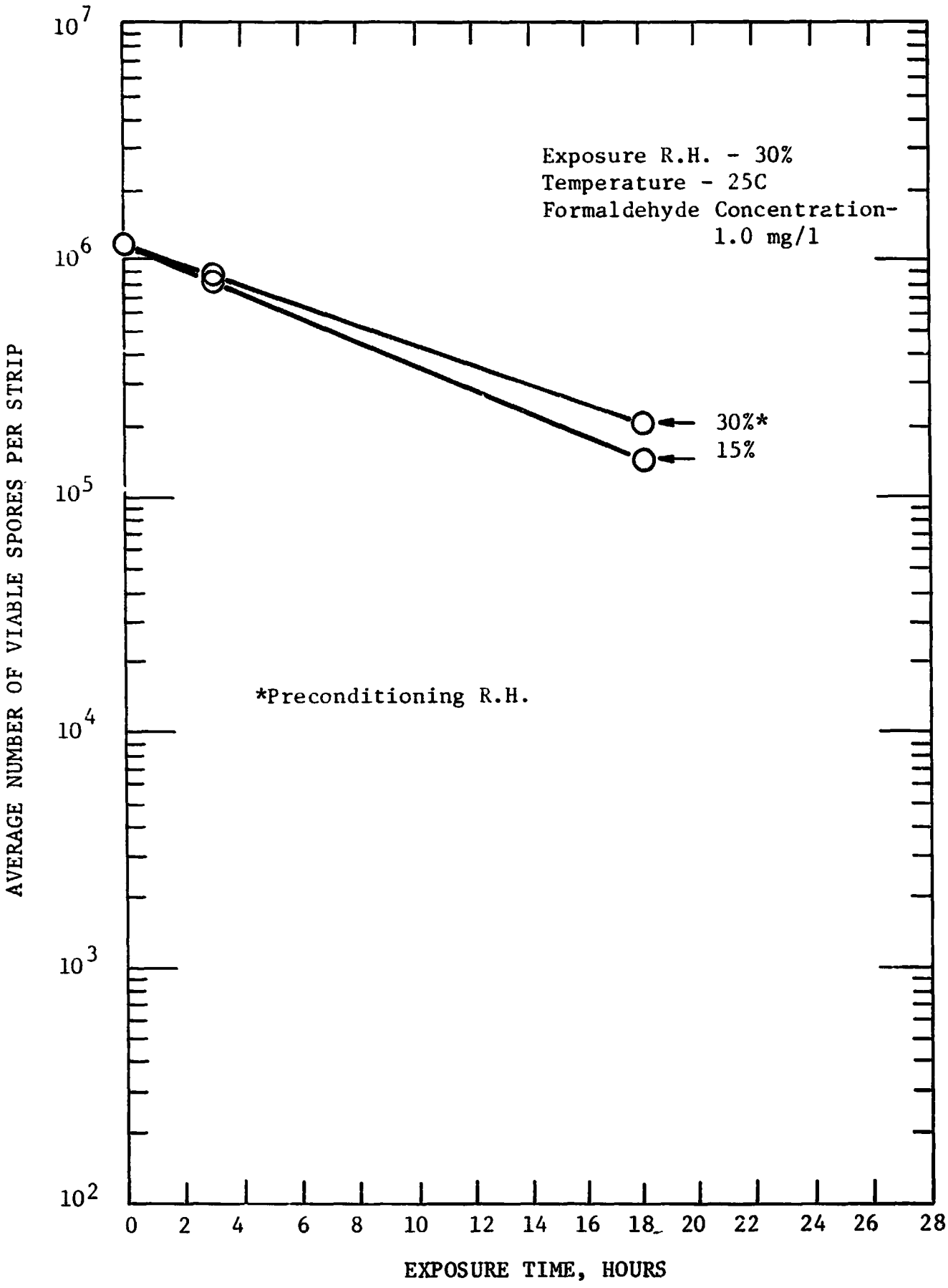


FIGURE 46: INACTIVATION OF Bacillus subtilis var. niger SPORES ON STAINLESS STEEL STRIPS

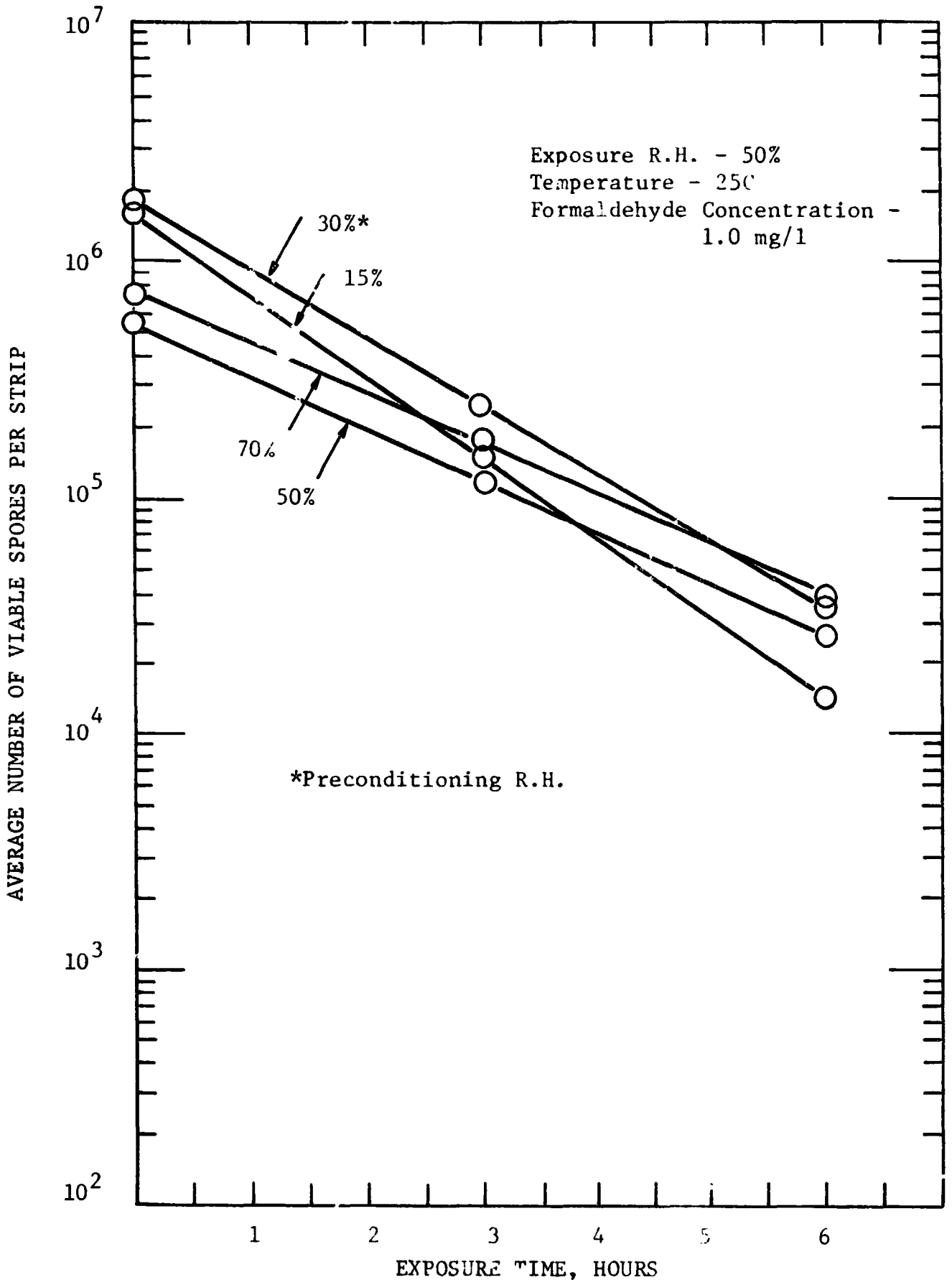


FIGURE 47: INACTIVATION OF Bacillus subtilis var. niger SPORES ON STAINLESS STEEL STRIPS

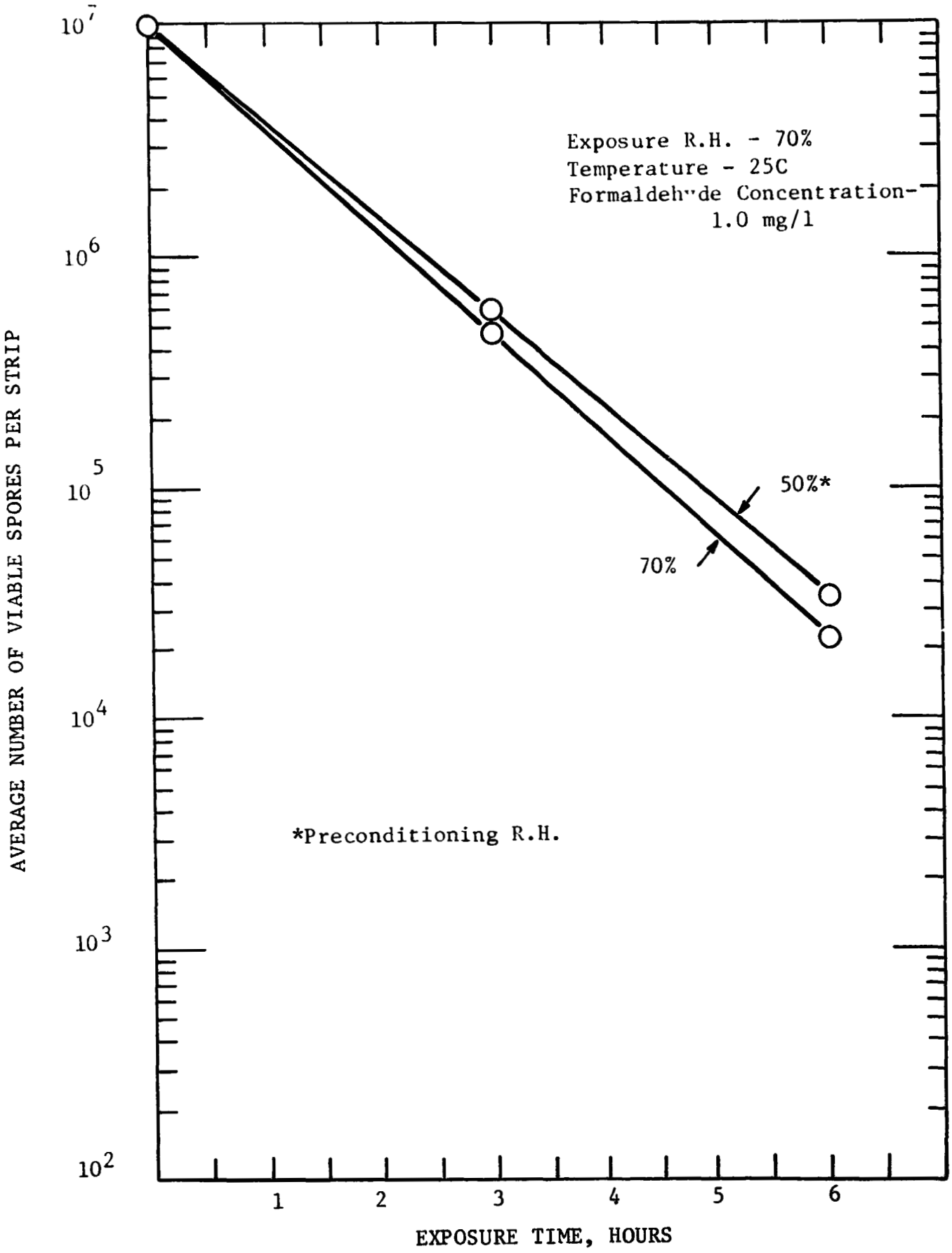


FIGURE 48: INACTIVATION OF Bacillus subtilis var. niger SPORES ON STAINLESS STEEL STRIPS

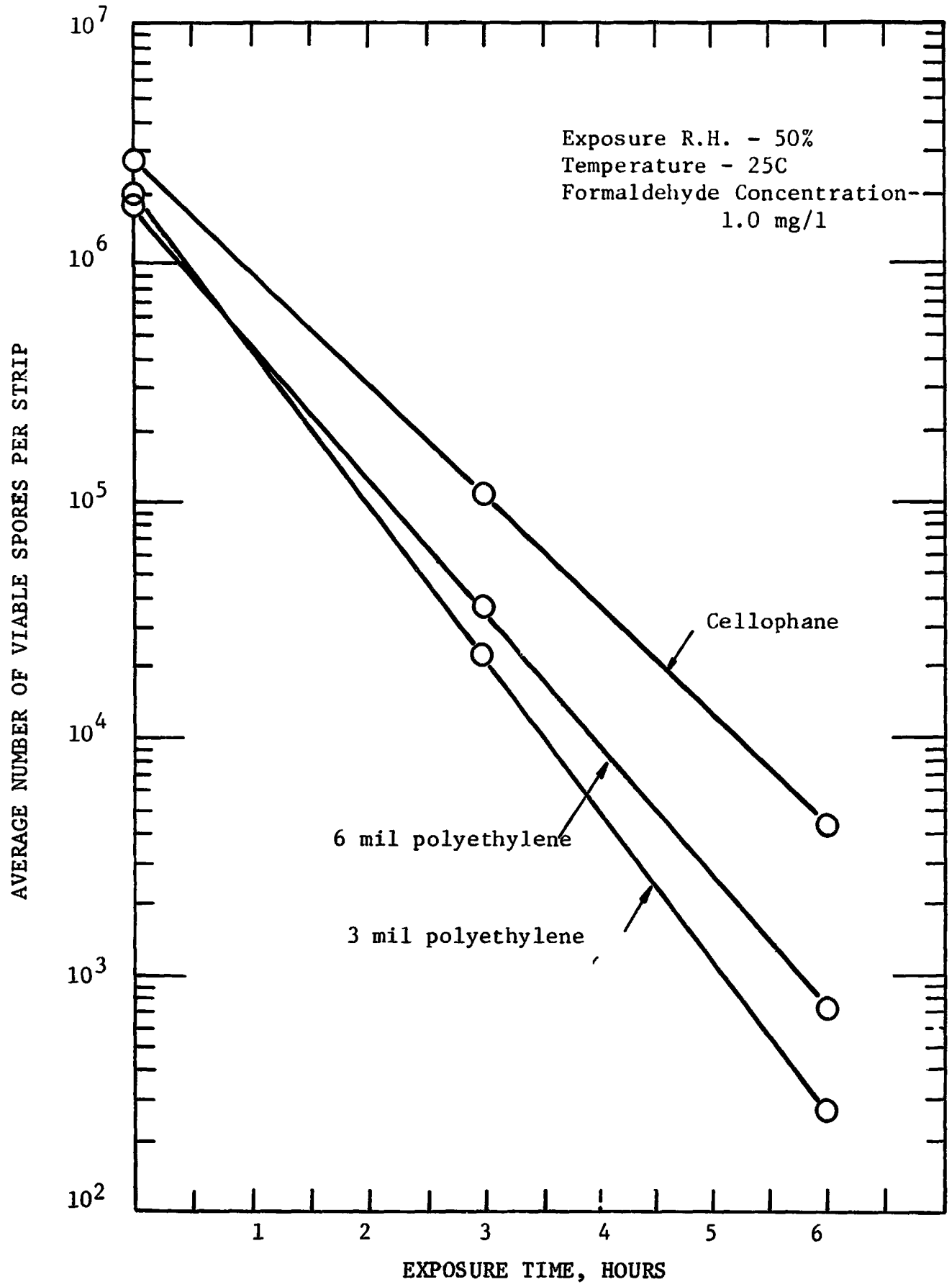


FIGURE 49: INACTIVATION OF BARRIER-ENCLOSED SPORES OF Bacillus subtilis var. niger

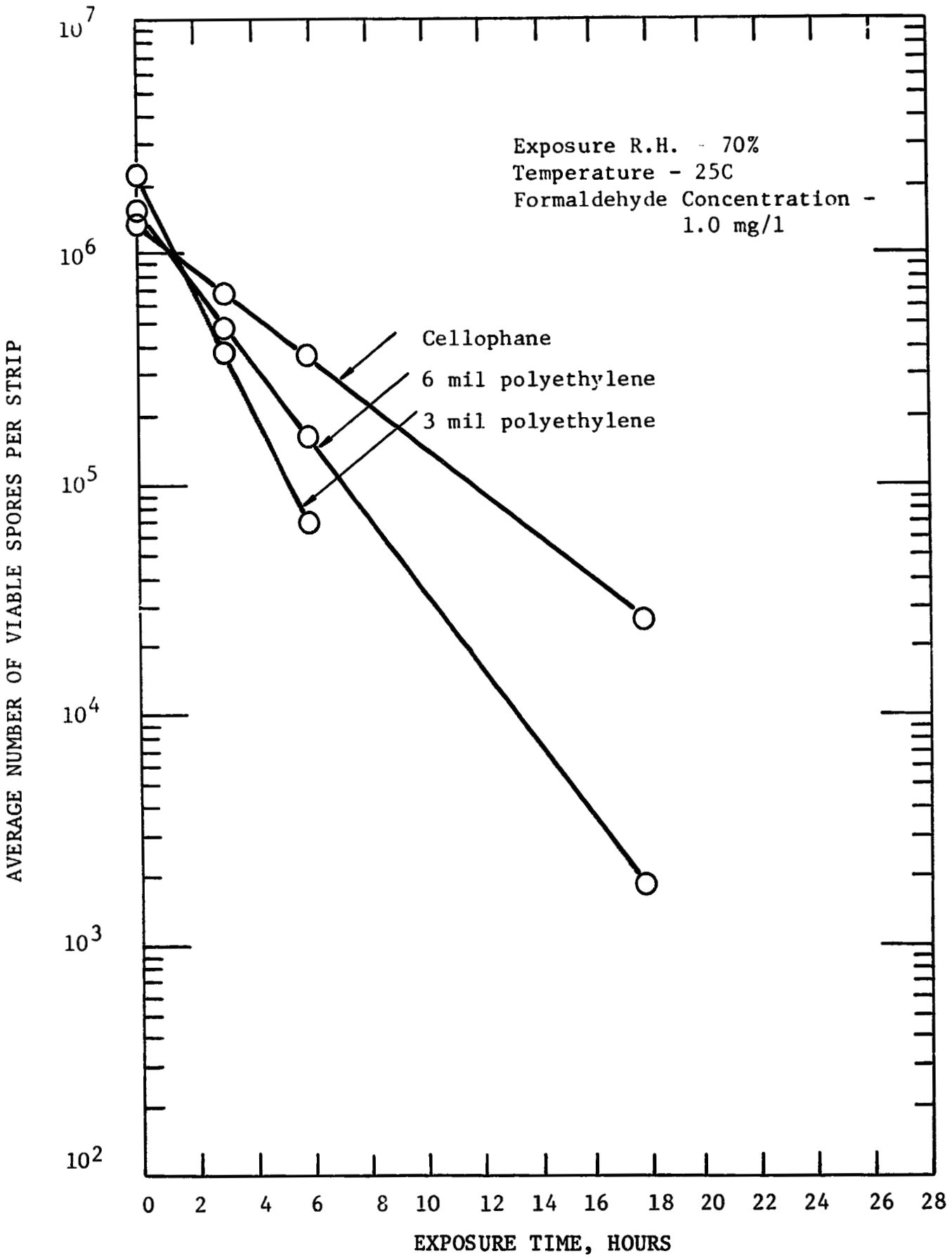


FIGURE 50: INACTIVATION OF BARRIER-ENCLOSED SPORES OF Bacillus subtilis var. niger

APPENDIX

APPENDIX

TABLE I

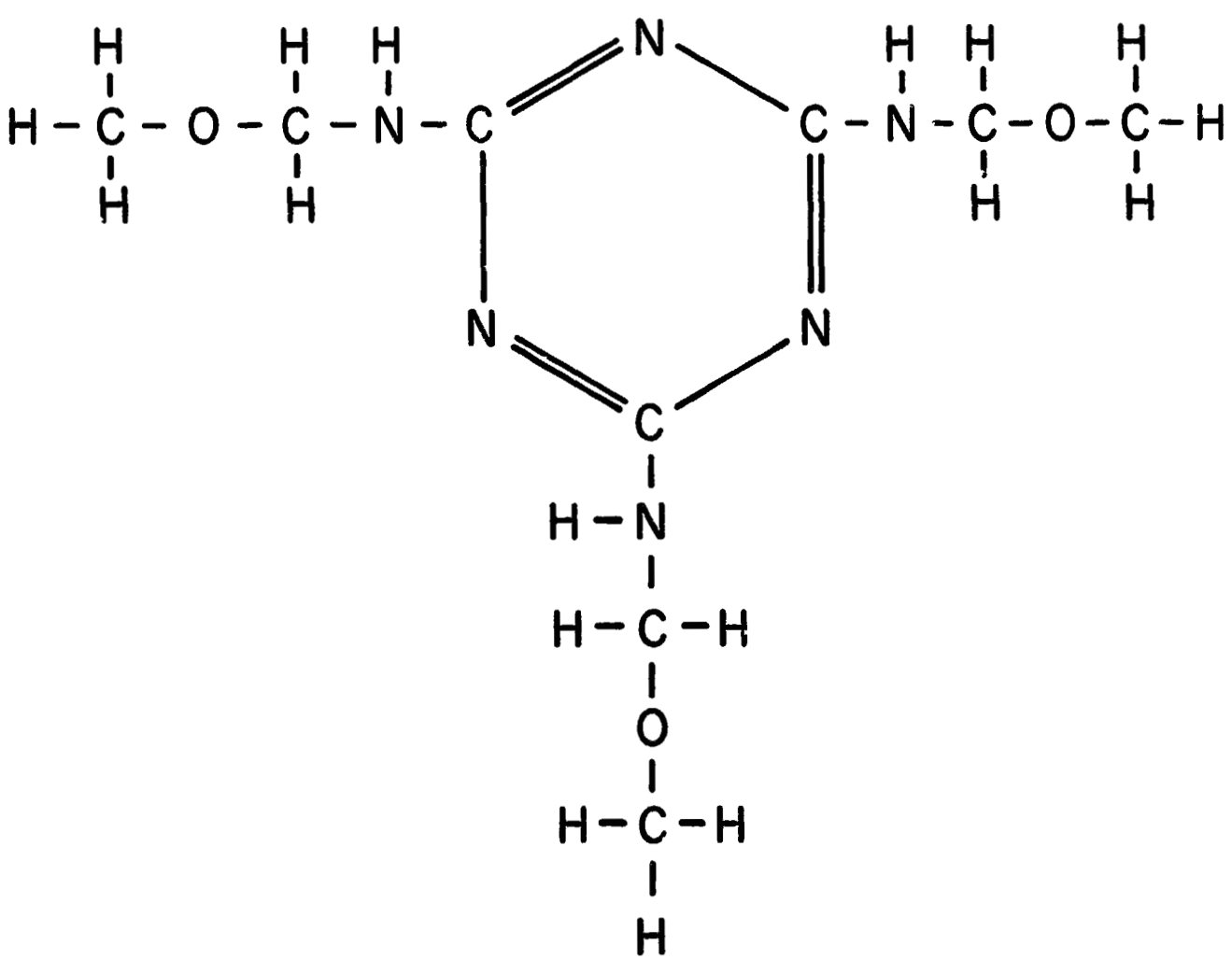
PROPERTIES OF PARAFORMALDEHYDE*

Assay, as formaldehyde, wt %, min	95.0
Ash, wt %, max	0.01
Iron, ppm by wt, max	2.0
Acidity, as formic, wt %, max	0.03
Water, wt %, max	5.0
Appearance	White solid
Odor	Pungent
Flash point, Tag open cup, F approx.	200
Ignition temperature, F approx.	575
Methanol content	None
Explosive limits for formaldehyde gas:	
Per cent by volume in air,	
Lower limit	7.0
Upper limit	73.0
Molecular weight, approx.	600

Solubility of paraformaldehyde is a function of pH and temperature; most soluble at pH extremes and reflux temperature.

* "Paraformaldehyde," Celanese Chemical Company, Division of Celanese Corporation, New York, New York (1957).

MELAMINE FORMALDEHYDE



APPENDIX

FIGURE 1: STRUCTURE OF MELAMINE FORMALDEHYDE

DIMETHYLOL UREA

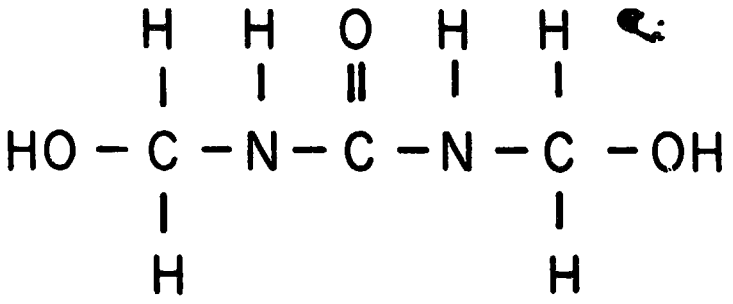
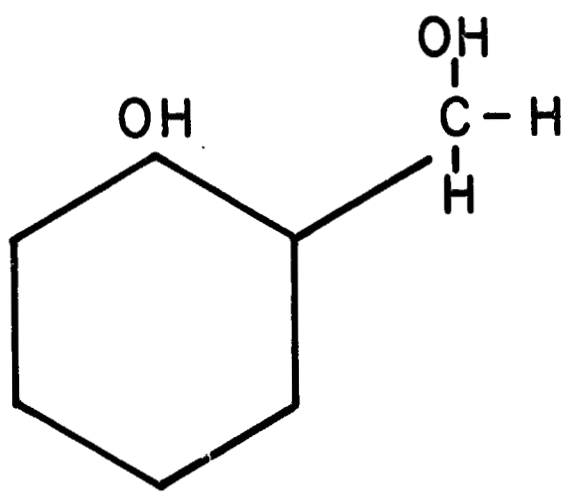


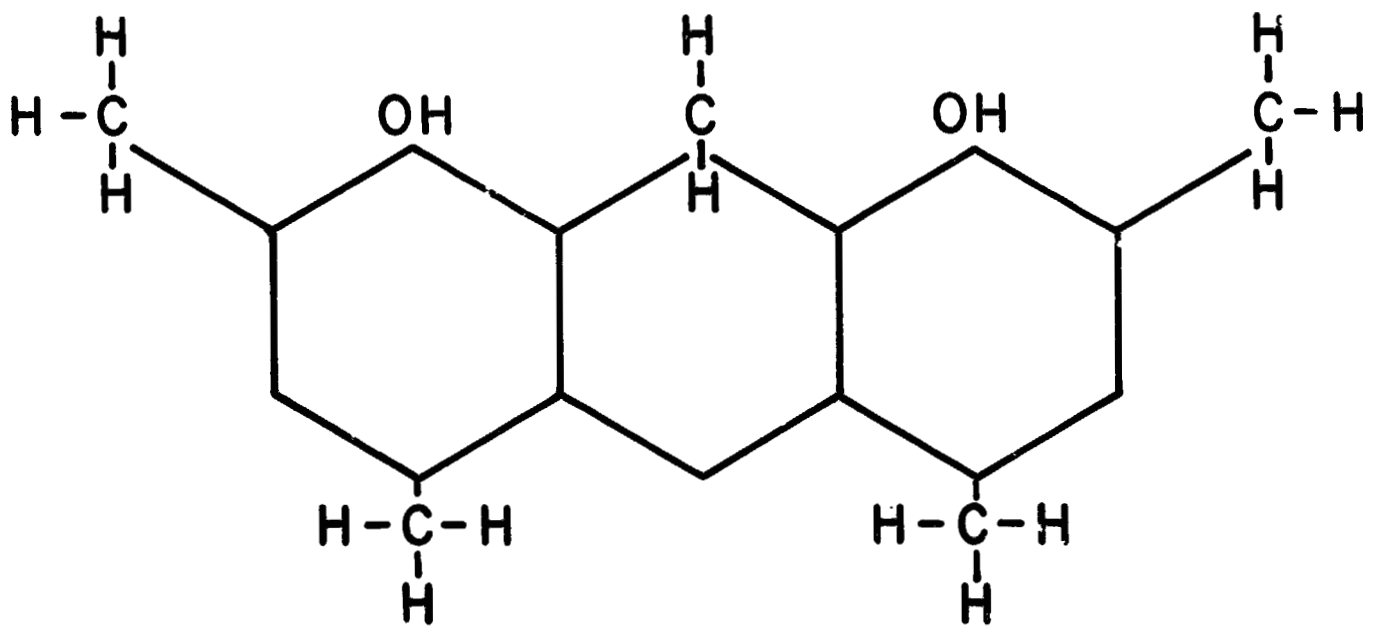
FIGURE 2: STRUCTURE OF UREA FORMALDEHYDE

PHENOL FORMALDEHYDE

ORTHO - METHYLOLPHENOL



METHYLENE PHENOL



APPENDIX

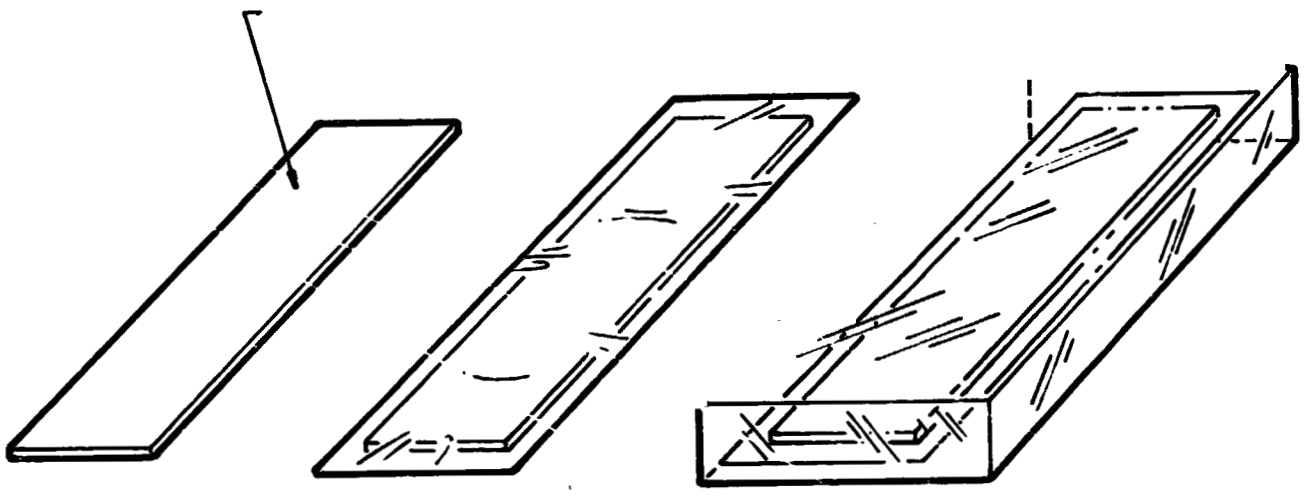
FIGURE 3; STRUCTURE OF PHENOL FORMALDEHYDE

PARAFORMALDEHYDE

$$HO-(CH_2O)_n-H$$

APPENDIX

FIGURE 4: STRUCTURE OF PARAFORMALDEHYDE



APPENDIX

FIGURE 5: EMBEDDING PROCEDURE FOR VERIFICATION OF INTERNAL STERILITY OF POTTING COMPOUND